JOURNAL OF ZOO AND AQUARIUM RESEARCH

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Review article

Marine mammal cognition and captive care:
A proposal for cognitive enrichment in zoos and aquariums

Fay E. Clark[1,2,*]

[1]*Centre for Animal Welfare, Royal Veterinary College, Hawkshead Lane, Hertfordshire AL9 7TA, United Kingdom*
[2]*Institute of Zoology, Zoological Society of London, London NW1 4RY, United Kingdom*
**Correspondence: fayelaineclark@hotmail.co.uk ; telephone: +44 (0) 1707 667045*

Keywords:
bottlenose dolphin, California sea lion, cetacean, cognition, cognitive enrichment, marine mammal, pinniped, well-being

Article history:
Received: 20 July 2012
Accepted: 17 December 2012
Published online: 24 July 2013

Abstract

Marine mammals, particularly cetaceans (whales, dolphins and porpoises) and pinnipeds (seals, sea lions and walruses) are popular in zoos and aquariums worldwide. Bottlenose dolphins and California sea lions have also been popular study species in animal cognition research since the 1950s, and many dolphin cognitive skills are on par with great apes. This paper proposes that 'cognitive challenges' can enhance the well-being of marine mammals, in line with previous studies on farm animals and great apes. While most captive marine mammals are trained and this challenges their social-cognitive skills to a moderate or high level, their physical-cognitive skills are not being challenged to a high level by floating 'toys' in the pool. This paper suggests that tasks originally developed to test the limits of dolphin and sea lion cognitive skill could be modified and implemented as 'cognitive enrichment' in zoos and aquariums. To be enriching, cognitive challenges should be relevant, motivating, controllable, and possible to master.

Introduction

Marine mammals are cetaceans (whales, dolphins and porpoises), pinnipeds (seals, sea lions and walruses), sirenians (manatees and dugongs), sea otters and polar bears (Rice 1998). Many thousands of marine mammals are housed in zoos and aquariums worldwide, the most popular being the bottlenose dolphin (*Tursiops truncatus*), and California sea lion (*Zalophus californianus*) (ISIS 2012). Hereafter, the bottlenose dolphin and California sea lion shall be referred to generically as dolphins and sea lions, and other species will be specifically stated.

To begin, this review explores the emerging branch of animal welfare science combining cognition and care, and suggests that 'cognitive challenges' can enhance animal well-being. Second, evidence that current marine mammal enrichment provides a low level of cognitive challenge will be discussed. Third, over half a century of cognitive research will be condensed, in order to summarise our current knowledge of marine mammal cognition. Finally, a new framework for marine mammal cognitive enrichment will be proposed, with suggestions on how this can be practically achieved in zoos and aquariums. The cognitive literature is expansive, and therefore we shall focus on studies of dolphins and sea lions which have taken place in the laboratory. More expansive reviews of marine mammal cognition can be found in Herman (2002a; 2010), Schusterman *et al.* (2002) and Jaakkola (2012).

A literature search was performed using Web of Science® citation database and Google Scholar. Boolean searches were performed for peer-reviewed papers, books, book chapters and conference proceedings using combinations of key terms such as 'marine mammal', 'cognition' and 'welfare'. The literature search spanned over four decades (1970 to present) owing to the long history of cognitive research on marine mammals.

Cognitive challenges for captive animals

Wild animals face many challenges that require the application of evolved cognitive skills (Meehan and Mench 2007). In contrast, captive animals tend to live in highly predictable and structured environments, and their cognitive skills may be challenged at a low or inappropriate level (Špinka and Wemelsfelder 2011). Fortunately, the provision of more cognitively challenging enrichment programmes for captive animals has increased over the last five years (Meehan and Mench 2007; Clark 2011). As Ross (2010 p.310) states, "Working to improve captive animal care without an understanding of how animal minds work, or the scope in which they perceive and interact with their social and physical environments, is akin to drawing a map without knowledge of any landmarks or bearings". In order to maximise well-being, animals should possess enough skill to master the challenge, and be motivated to do so (Meehan and Mench 2007). Clark (2011 p.6) incorporated these thoughts into the following definition of cognitive enrichment: "…a task (or tasks) whose use (1) engages evolved cognitive skills by providing opportunities to solve problems and control some aspect of the environment, and (2) is correlated to one or more validated measures of wellbeing".

Cognitive challenges have benefitted animal well-being on the farm, and more recently in the zoo. On the farm, Ernst *et al.* (2005) and Puppe *et al.* (2007) challenged pigs with a discrimination task several times a day in their normal social group. Pigs exposed to this challenge over a ten-week period exhibited a significant reduction in abnormal behaviour (Puppe *et al.* 2007). Langbein *et al.* (2009) also found that dwarf goats (*Capra hircus*) continued to solve a previously learnt cognitive problem without an external reward. Although it was a cognitive study with no focus on well-being, Mackay (1981) similarly found that dolphins trained to whistle at a specific frequency in order to activate a food dispenser continued to do so without a food reward. Independently learning the solution to a task can have a positive impact on well-being, as shown in domestic cows (Hagan and Broom 2004). Clark *et al.* (in review) showed that when zoo-housed chimpanzees (*Pan troglodytes*) interacted with a maze-like cognitive challenge device, they displayed increased 'competence' (prolonged exploration of the device including innovative problem-solving strategies) and 'agency' (exploration of the wider captive environment, and social play).

Marine mammal well-being and enrichment in captivity

Behavioural problems

The most commonly reported behaviour of concern in captive marine mammals is repetitive swimming in a fixed pattern around the pool (e.g. cetaceans: Gygax 1993; Sobel *et al.*, 1994; pinnipeds: Hunter *et al.* 2002; Smith and Litchfield 2010). However, a more recent study by Miller *et al.* (2011) found the repetitive swimming across six dolphin facilities was low (less than 5% scans). Although this type of swimming fits the definition of stereotypical behaviour because it is unvarying and repetitive (Mason 1991), it may also have an obvious function. Continuous swimming around the pool could be interpreted as functional patrolling behaviour, because the 'outer patrol border' of the pool never changes. Furthermore, stereotypical behaviour is linked to good or neutral well-being nearly as often as poor well-being (Mason and Latham 2004). Another behaviour which may or may not be problematic in dolphins is surface-directed behaviour. Galhardo *et al.* (1996) found that captive dolphins spent a disproportionate amount of time with their heads above water. This is not a particularly natural behaviour for dolphins (similar to repetitive swimming as discussed above) but could be interpreted as functional because most environmental complexity is on or above the water-surface (e.g. the presence of animal staff, floating enrichment objects).

Other reported behavioural problems in cetaceans are low and come from outdated publications; for example repeated head-pressing against the wall (Greenwood 1977), bumping the head against the floor while swimming (Amundin 1974), and excessive aggressive or sexual activity (Sweeney 1990; Samuels and Spradin 1995). In pinnipeds, flipper chewing, excessive self-grooming and body-scratching against hard surfaces have been reported (Kastelein and Wiepkema 1989; Smith and Litchfield 2010). These self-directed and injurious behaviours are widely accepted as signs of compromised well-being (Moberg and Mench 2000). In summary, data are lacking on the aetiology of problem behaviours in cetaceans and pinnipeds. A survey of current behaviour is needed to establish the form and function of behaviours, an objective assessment of whether they are problematic, and a measure of how intensely they have been studied.

Enrichment

Marine mammal exhibits in zoos and aquariums are usually smooth-edged concrete pools with glass windows. This design helps to achieve the highest safety and hygiene standards (Joseph and Antrim 2010), but is also criticised for being sterile (Rose *et al.*, 2009). Indeed, it is surprising that although enrichment could be used to compensate for a lack of structural exhibit complexity, it is usually simplistic. A literature search yielded twelve peer-reviewed papers and book chapters on cetacean and pinniped enrichment (not including 'social' enrichment provided by training). Within this small amount of published literature, there is a clear trend for using plastic and rubber enrichment 'toys' that float on the surface of the water (e.g. cetaceans: Gewalt 1989; Kuczaj *et al.* 2002; Delfour and Beyer 2011; pinnipeds: Grindod and Cleaver 2001). These studies show that, as a general rule, floating objects rouse immediate interest and interaction but are quickly habituated to. Submerged objects have been used far less (weather vane: Amundin 1974; fire hose: Amundin 1974; Berglind 2005).

The most advanced dolphin enrichment study thus far was by Berglind (unpublished thesis, 2005), who ran water through submerged fire hose so that it moved erratically, subsequently triggering group hunting displays and increased sonar activity. For pinnipeds, Kastelein and Wiepkema (1989) recognised that walruses (Odobenus rosmarus) had a lack of naturalistic foraging opportunities, and decreased repetitive swimming behaviour by providing buried food. However at large, the specific characteristics of objects which make them effective enrichment for marine mammals (for example buoyancy, destructibility and complexity) have been largely unexplored.

Despite the fact that most captive marine mammals live in physically un-complex environments (especially under the water's surface), most species have their social cognitive skills challenged to a moderate or high level via training and less formal interactions with humans. Positive reinforcement training is an integral part of marine mammal management (Brando 2010), and is thought to be enriching for captive animals because it increases an animal's sense of control in the environment (Laule and Desmond 1998). Repetitive swimming in Steller sea lions (*Eumetopias jubatus*) was significantly decreased by incorporating training into their management routine (Kastelein and Wiepkema 1988). However, it is not clear whether increased human contact or the training process itself was beneficial to the subjects. Human-dolphin swim programmes have become increasingly popular in zoos and aquariums worldwide (Miller *et al.* 2011), but evidence for whether these programmes are enriching is mixed. For example, a multi-facility study (Miller *et al.* 2011) provided convincing evidence that swim programmes were enriching because they were associated with increased dolphin play and behavioural diversity (including swimming style). Conversely, swim programmes that are less structured, for example where visitors can swim freely with the dolphins for a period of time, have been associated with avoidance of, or aggression towards, humans (Samuels and Spradin 1995; Kyngdon *et al.* 2003).

Cognitive skills of dolphins and sea lions

History of study

Whether marine mammals are 'too intelligent for captivity' is a topic of on-going scientific and public debate that will not be discussed here (see Rose *et al.* 2009; Grimm 2011; Marino and Frohoff 2011). However, this paper will condense the evidence taken from over half a century of cognitive study in the laboratory and field (Schusterman and Kastak 2004; Pack 2010), that dolphins and sea lions have high cognitive skills. The dolphin has been of particular interest to cognitive scientists because it has an exceptionally large brain relative to body size (Marino 2002), a high level of 'intelligence' comparable to humans and great apes (Marino 2002), and is relatively easy to manage in shallow pools (Herman 2002a). There is less cognitive research on the sea lion, but significantly more so than any other pinniped species. Similar to the dolphin, the sea lion has high 'intelligence', is easy to train, and adapts well to living in shallow pools (Schusterman *et al.* 2002). Furthermore, many of the methods used to test dolphin cognition have been transferable to sea lions (Schusterman and Kastak 2004).

Dolphin echolocation

Echolocation in dolphins refers to their ability to locate and discriminate underwater obstacles, by producing high-frequency sound waves and receiving the echoes that reflect off these obstacles (Au 1993). In contrast to dolphins, sea lions do not echolocate (Schusterman *et al.* 2000). Echolocation was discovered in captive dolphins in the 1950's and has since been studied rigorously by concealing underwater objects in black boxes, and by placing rubber cups over dolphins' eyes to prevent them using normal sight (reviewed by Au 1993). Not only can dolphins use echolocation to recognise three-dimensional shapes, they can also correctly match a shape inspected through echolocation with an identical shape inspected through vision and vice versa (Pack *et al.* 2004). Furthermore, dolphins can 'eavesdrop' on the echolocation of conspecifics and use this to recognise objects (Xitco and Roitblat 1996).

Concept formation

'Concepts' are general rules that animals apply to the novel problems they encounter in life (Schusterman and Kastak 2004). These concepts can be abstract (e.g. same/different), relational (e.g. larger/smaller) and perceptual (e.g. food/non-food). Concept formation in marine mammals has been tested in the laboratory using an experimental method called matching-to-sample (MTS), where a subject is shown a 'sample stimulus' and reinforced (for example with a fish) for choosing the correct stimulus from two or more 'comparison stimuli'. Dolphins have shown that they understand the concept of larger/smaller quantities (Jaakkola *et al.* 2005), and Abramson *et al.* (2011) recently demonstrated that South American sea lions (*Otaria flavescens*) could assess and select the larger of two sets of quantities.

Being able to recognise objects when they have been rotated in three dimensions is another useful skill for animals living in complex environments. Tests of mental rotations on marine mammals have been undertaken using the MTS method described above, where a subject must match an object with its mirror image or rotation. Studies on dolphins (Herman *et al.* 1993), beluga whales (*Delphinapterus leucas*; Murayama and Tobayama 1995) and sea lions (Mauck and Dehnhart 1997) show that marine mammals can recognise rotated objects, but similar to humans, the time it takes them to do so is positively correlated to the degree of rotation.

Learning and memory

Dolphins and sea lions have excellent short-term memory for sights and sounds (Herman 2002a; Schusterman *et al.* 2002). In 'delayed' MTS tests, the time delay between presentation of the sample stimulus and comparison stimuli is increased to find the maximum length of time a subject can retain the memory of the sample stimulus. Not only can dolphins remember specific sounds, one dolphin could remember lists of up to four novel sounds, compared to a maximum of seven in humans (Thompson and Herman 1977).

Dolphins also possess good spatial memory; in other words memory for the spatial location of events or objects. Thompson and Herman (1981) played underwater sounds in one of four pool locations, and a dolphin correctly swam to the location of the sound after a time delay of up to 70 seconds. Jaakkola *et al.* (2010) found that like many terrestrial animals, dolphins can track the spatial location of visible objects, but in contrast to great apes they cannot track the spatial location of hidden objects. This is probably because dolphins rely on their echolocation to identify objects hidden from sight (Jaakkola 2012).

Understanding symbols and televised images

Following in the footsteps of great ape research, 'artificial languages' were taught to captive dolphins in the 1980's (reviewed by Herman 2002a; Jaakkola 2012). These languages contained symbols for objects (e.g. ball), their positions in the pool (e.g. left), actions (e.g. jump over) as well as questions relating to objects (e.g. is the object in the pool?). Similar to human language, symbol order changed meaning; for example 'take the ball to the bucket' is different from 'take the bucket to the ball'. Dolphins appear to understand both semantics (meaning) and syntax (order) of symbols (reviewed by Herman 2002a), and studies on sea lions have yielded comparable results (Schusterman and Kastak 2004). The ability of dolphins and sea lions to correctly answer 'yes/no' questions (e.g. is the ball in the pool?) is particularly impressive, and is achieved by the subject pressing one paddle for 'yes' and another for 'no' (Herman and Forestell 1985; Schusterman *et al.* 2002)

Television screens have been used to test whether marine mammals understand external representations of objects and actions. Thus far, dolphins have followed commands given to them by humans on a television screen, imitated televised dolphins, and correctly responded in MTS trials when the sample stimuli are presented on-screen (Herman 2002). In fact, the immediate, spontaneous response of dolphins to televised images outperforms that of chimpanzees, who respond correctly to televised images only after intensive training (Savage-Rumbaugh 1986).

Awareness of self and others

The large brains of dolphins (and great apes) probably evolved due to strong social forces (reviewed by Marino *et al.* 2007), and therefore both taxa have a good understanding of themselves and others. The traditional method of testing whether large-brained animals recognise themselves (i.e. have self-awareness) is through the mirror self-recognition mark test (Gallup 1970). According to this test, great apes and some other primates, elephants, cetaceans, and corvids (crows) are the only nonhuman animals that are self-aware (reviewed by Herman 2012). The evidence is fairly unequivocal in dolphins (Reiss and Marino 2001); killer whales (*Orcinus orca*) and false killer whales (*Pseudorca crassidens*) could also be self-aware (in contrast to sea lions) because they appear to look significantly more at a body part in the mirror if the body part has been marked with a spot (Delfour and Marten 2001).

In a further examination of self-awareness, Mercado *et al.* (1998) found that a dolphin could repeat (imitate) its previous behaviour on command. In order to perform imitative behaviour, an animal must have a mental representation of others and/or itself; therefore imitation is regarded as a very high-level cognitive ability. Dolphins and sea lions are known to be excellent imitators of both actions and vocalisations, of both conspecifics and humans, often doing so spontaneously (reviewed by Herman 2002b). It is particularly impressive that dolphins and sea lions can imitate a human's motor actions despite having vastly different body shapes, interpreting for example that their fins are equivalent to human arms. Dolphins and beluga whales are capable of mimicking human sounds (Herman 2002b; Ridgway *et al.* 2012) and interestingly, a captive harbour seal could apparently mimic ten human words (Ralls *et al.* 1985).

In addition to imitation, dolphins possess a range of cognitive skills related to the understanding of other's actions, known as 'joint attention' (reviewed by Pack and Herman 2007). In fact, some of the joint attention abilities of dolphins exceed those shown by great apes. Dolphins are one of few non-domesticated species that understand the meaning of human pointing without prior training, and can determine which object a person is gazing at (Pack and Herman 2007). Furthermore, Xitco *et al.* (2004) found that dolphins pointed (oriented) their bodies toward an object, while looking to see if they had the attention of a human.

Planning and problem-solving

The ability to create appropriate plans when confronted with novel problems has obvious survival advantages, but has been demonstrated in relatively few species. Examples of planned behaviour in wild cetaceans are numerous, and include the cooperation of male dolphins when mating (Connor 2007), cooperation of killer whales when hunting (Visser *et al.* 2008), and dolphins using sponges to protect their rostrums while foraging in sharp rocks (Smolker *et al.* 2007). Incidentally, sponge-use is the only reported example of tool use in wild cetaceans, in contrast to more numerous accounts in captivity (see Jaakkola 2012).

Kuczaj *et al.* (2010) examined how two dolphins planned their behaviour when faced with a novel underwater task. The task required dolphins to transport and drop four weights into a compartment in order to release a fish reward. The task was demonstrated to the dolphins by a diver, using one weight at a time. Interestingly, however, dolphins chose to carry multiple weights to the apparatus rather than simply imitating the diver's actions. The same dolphins were presented with a new task requiring the placement of weights into three compartments, and were able to use the weights in the most economical manner to maximise the quantity of food reward (Kuczaj *et al.* 2010). Dolphins can also be commanded to produce novel behaviours (Herman 2006), suggesting they possess another rare trait in the animal kingdom: creativity. Herman (2006) also commanded a pair of dolphins to create a novel behaviour at the same time, which they were able to do successfully. No other animal species tested, other than humans, has revealed the capacity for synchronous creative behaviour at such an accomplished level (Herman 2010).

Marine mammal cognition and captive care

A cognitive enrichment framework for marine mammals

So far, this review has summarised why dolphins and sea lions are highly intelligent animals, with dolphins being on par with (or exceeding) great apes based on the cognitive tests provided thus far. However, it is doubtful that the physical cognitive skills of sea lions and dolphins are being challenged to a high level in the clean, smooth-sided concrete and glass pools usually found in zoos and aquariums. Hitherto, floating 'toy' objects have provided more complexity above the water-surface than below. For dolphins, this type of enrichment does not make full use of their echolocation; their most sophisticated and unique cognitive ability.

This paper now culminates with a new proposal for marine mammal cognitive enrichment. In this framework, previous laboratory cognitive tests are examined; their principles are deconstructed; and then reconstructed to create cognitive challenges practical for zoos and aquariums. Of course, it is important to evaluate the effect of cognitive challenges on well-being before they can be characterised as enriching, and this procedure will be discussed in due course. First, three examples of cognitive challenge will be suggested, with a particular emphasis on challenging physical cognitive skills. The first approach requires a protracted period of animal training, while the other two approaches rely on giving subjects opportunities to solve challenges for themselves with minimum human intervention.

(i) Stimulus matching: control over the environment

When animals are given increased choice and control over their environment, this can lead to enhanced well-being (Laule and Desmond 1998). The first suggested approach to cognitive enrichment is to allow subjects more control by participating in discrimination trials. Both dolphins and sea lions have been tested using MTS procedures for many decades, and have demonstrated a strong understanding of symbols (Schusterman and Kastak 2004). Using this procedure in a zoo or aquarium setting, visual or acoustic cues are used to summon subjects to various reward stations. Then, subjects are given the opportunity to correctly discriminate between different visual stimuli to receive a reward (Ernst *et al.* 2005; Puppe *et al.* 2007). To take advantage of their multi-modal natures, underwater tones will be played (Kuczaj *et al.* 1998), thus requiring animals to touch corresponding symbols displayed on wooden boards (see von Fersen *et al.* 2000) or television screens (see Delfour and Marten 2006), placed in or near to the pool.

By voluntarily participating in these tasks, subjects will gain more control over their environment (Langbein *et al.* 2009) and potentially benefit from their own learning success over time (Hagan and Broom 2004). The reward could be food, access to objects, or more control over resources such as fountain water or lighting. This is no doubt a labour-intensive method of providing cognitive challenge, but it capitalizes on the strong bond already developed between marine mammals and their trainers. In fact, Herman (2002a) suggests that teaching subjects (i.e. physical demonstration of the relationships between objects and symbols) may be more efficient than training. The equipment for this approach will be low cost, requiring at minimum a set of underwater speakers and wooden display boards, and a trainer to reinforce subjects for correct responses (whereas incorrect responses will be ignored). If funding permits, this approach could also incorporate the use of computer touch screens (Delfour and Marten 2006) and automatic feeders (Mackay 1981).

(ii) Submerged problem-solving devices

The second approach to cognitive challenge uses submerged 'problem-solving devices', temporarily secured to the pool wall (Clark *et al.* In review). This was alluded to, four decades ago by Amundin (1974), who suggested (but never formally evaluated) textured 'structure plates' attached to the walls of marine mammal pools. Kuczaj *et al.* (2010) recently found that dolphins efficiently planned their responses to a submerged physical task, but little work on dolphin problem-solving has been attempted since. In particular, visually hidden challenges will stimulate dolphin echolocation and provide a more acoustically rich environment than concrete pool walls. Current research on cognitive challenge devices for dolphins suggests they are highly motivated to interact with devices, even in the absence of a food or other external reward (Clark, personal observation).

Ideally, a cognitive challenge device requires very little human intervention in terms of demonstration or providing food rewards. At any one time, several marine mammals in an exhibit may be off-show in holding pools (Joseph and Antrim 2010), and cognitive challenge devices could be particularly useful in these situations to prevent boredom or distract from social tensions. Furthermore, if a cognitive challenge device is modular, virtually hundreds of versions of the device can be built and implemented over time, therefore varying task complexity and sustaining task novelty. This is a relatively low-cost approach to cognitive challenge, because the component modular pieces of the device can be re-used over time.

(iii) Submerged spatial problem-solving tasks

The third and final approach is an extension of the second approach, and uses problem-solving tasks over a larger area to challenge spatio-cognitive skills. This takes inspiration from dolphin cognition studies undertaken at The Seas facility (Disneyworld, Florida), where the public can watch dolphins participating in cognitive tests in their normal pool (Harley *et al.* 2010). The approach is to create a cognitively challenging 'obstacle course' in the centre of the pool. A number of tasks are laid out and the rate or method of completion by subjects will affect the level of reward gained.

Spatial problems are potentially more naturalistic (and therefore motivating) to dolphins and sea lions than static devices attached to the pool wall. This approach also requires little or no training, and the cost of equipment can be low (e.g. Perspex tubes containing weights) or high (e.g. fully automated underwater keyboards; see Harley *et al.* 2010). It may be economical to build a number of permanent connection points in the pool where task components can be temporarily attached when needed.

Limitations in the zoo and aquarium environment

There are of course several caveats when providing cognitive challenges to marine mammals. First, it is important to acknowledge that most cognitive data discussed in this paper have been collected on a few 'well-schooled' individuals (Herman 2002a Pack 2010) and therefore generalisation to other individuals should be conservative. It is unrealistic to expect the average captive dolphin or sea lion to spontaneously exhibit the same level of cognitive skill as the handful of laboratory subjects. Rather, we should recognise the cognitive potential of each subject, and try to cultivate this potential over time.

A note on health and safety is also necessary. Often, marine mammals pick up, manipulate and intentionally or unintentionally ingest objects that fall into their pools (Joseph and Antrim 2010). Notably, large air-filled balls can lodge in an animal's throat when diving, and tethering objects can also be dangerous because this can result in entanglement (Joseph and Antrim 2010). All the necessary precautions should be taken; an observer should always be present when apparatus are used in the pool, particularly when they are submerged or contain moving parts.

Evaluating cognitive challenges

Re-visiting Clark's definition of cognitive enrichment (Clark 2011), a cognitive challenge must have a positive impact on well-being before it can be called enrichment. Unfortunately, this may be easier said than done in marine mammals, because fundamental research is lacking on indicators of positive and negative well-being. However, there are several guiding principles we can take from cognitive challenge research on other animals. First, if the level of challenge is relevant or un-motivating (i.e. does not suit an animal's anatomy, natural history and cognitive skill level), apathy, boredom or frustration may be observed (Meehan and Mench 2007). Second, control and mastery of a cognitive challenge are important for well-being (Meehan and Mench 2007). Herman (2002a p.276) suggests that "When introducing new (cognitive) problems, we take care that they are at an initial level likely to lead to success, and then increase the difficulty and complexity at a rate or in contexts that tend to promote overall success".

Conclusions

Dolphins possess complex cognitive skills largely on par with (and sometimes exceeding) great apes. Sea lions also perform strongly on many of the same laboratory cognitive tests. Both dolphins and sea lions have good short and long term memory skills, an understanding of abstract concepts and symbols, and creative problem-solving abilities. It is therefore surprising that previous attempts at enrichment have provided little opportunity to apply these skills to solve complex physical problems. By providing floating objects as enrichment, we are barely catering for the high cognitive skills of marine mammals.

With over five decades of dolphin and sea lion cognitive study at their disposal, marine mammal carers are in a strong position to provide more appropriate, relevant enrichment. This review suggests that 'cognitive challenges' should be provided to marine mammals as a form of enrichment; these challenges can range from small match-to-sample discrimination trials, to large underwater obstacle courses. To some extent, cognitive challenges might help offset the fundamental limitations of housing large-brained aquatic mammals in sterile pools. The opportunities for cognitive challenge design are endless, and inspiration is available from the cognitive literature. This paper aims to inspire further developments in marine mammal enrichment, and encourages more debate on the link between cognition and care in large-brained animals.

Acknowledgements

The author wishes to thank Christopher Wathes, Anthony Sainsbury and Stan Kuczaj for discussions leading to the ideas presented in this review.

References

Abramson J.Z., Hernández-Lloreda V., Call J., Colmenares F. (2012). Relative quantity judgments in South American sea lions (*Otorio flovescens*). *Animol Cognition* 14: 695-706.

Amundin M. (1974). Occupational therapy for harbour porpoises, *Phocoeno phocoeno*. Aquatic Mammals 2: 6-11.

Au W.W.L. 1993. The sonar of dolphins. Springer-Verlag, New York.

Berglind M. (2005). *Acoustic enrichment for dolphins in pool environments.* Unpublished Masters thesis. Sweden: Linköping University.

Brando S. (2010). Advances in Husbandry Training in Marine Mammal Care Programs. *International Journal of Comparative Psychology* 23: 777-791.

Clark F.E., Hitchcock D., Sainsbury A.W., Wathes C.M. *In review.* Can cognitive challenges be enriching? Evaluation of a novel cognitive challenge device for zoo-housed chimpanzees (*Pan troglodytes*).

Clark F.E. (2011). Great Ape Cognition and Captive Care: Can Cognitive Challenges Enhance Psychological Well-being? *Applied Animal Behaviour Science* 135: 1-12.

Connor R.C. (2007). Dolphin social intelligence: complex alliance relationships in bottlenose dolphins and a consideration of selective environments for extreme brain size evolution in mammals. *Philosophical Transactions of the Royal Society B* 362: 587-602.

Delfour F., Beyer H. (2012). Assessing the effectiveness of environmental enrichment in bottlenose dolphins (*Tursiops truncotus*). *Zoo Biology* 31: 137-150.

Delfour F., Marten K. (2006). Lateralized visual behavior in bottlenose dolphins (*Tursiops truncatus*) performing audio–visual tasks: The right visual field advantage. *Behavioural processes* 71: 41-50.

Delfour F., Marten K. (2001). Mirror image processing in three marine mammal species: Killer whales (*Orcinus orco*), false killer whales (*Pseudorco crassidens*) and California sea lions (*Zolophus californianus*). *Behavioural Processes* 53: 181-190.

Ernst K., Puppe B., Schon P.C., Manteuffel G. (2005). A complex automatic feeding system for pigs aimed to induce successful behavioural coping by cognitive adaptation. Applied Animal Behaviour Science 91: 205-281.

Galhardo L., Appleby M.C., Waran N.K., dos Santos M.E. (1996). Spontaneous activities of captive performing bottlenose dolphins (*Tursiops truncotus*). *Animal Welfare* 5: 373-389.

Gallup G.G. Jr. (1970). Chimpanzees: Self recognition. *Science* 167:86-87.

Gewalt W. (1989). Orinoco freshwater dolphins (*Inio geoffrensis*) using self-produced air bubble rings as toys. *Aquotic Mammols* 15: 73-79.

Goldblatt A. (1993). Behavioural needs of captive marine mammals. *Aquotic Mammals* 5: 15-17.

Greenwood A.G. (1977). A stereotyped behavior pattern in dolphins. *Aquatic Mommals* 5: 15-17.

Grimm D. (2011). Are dolphins too smart for captivity? *Science* 332: 526-529.

Grindrod J.A.E., Cleaver J.A. (2001). Environmental enrichment reduces the performance of stereotypic circling behaviour in captive common seals (*Phoca vitulina*). *Animol Welfore* 10: 53-63.

Gygax L. (1993). Spatial movement patterns and behaviour of two captive bottlenose dolphins (*Tursiops truncatus*): absence of stereotyped behaviour or lack of definition? *Applied Animal Behaviour Science* 38: 337-344.

Hagan K., Broom D.M. (2004). Emotional reactions to learning in cattle. *Applied Animol Behoviour Science* 85: 203-213.

Harley H.E., Fellner W., Stamper M.A. (2010). Cognitive Research with Dolphins (Tursiops truncatus) at Disney's The Seas: A Program for Enrichment, Science, Education, and Conservation. *Internotionol Journol of Comporotive Psychology* 23: 331-343.

Herman L.M. (2002a). Exploring the cognitive world of the bottlenosed dolphin. In: Bekoff M., Allen C., Burghardt G. (eds) *The cognitive onimol: Empiricol and theoreticol perspectives on onimol cognition*. MIT Press, Cambridge. pp. 275-283.

Herman L.M. (2002b). Vocal, social, and self-imitation by bottlenosed dolphins. In: Nehaniv C. and Dautenhahn K. (eds) *Imitotion in Animols ond Artifocts.* MIT Press, Cambridge. pp. 63-108.

Herman L.M. (2006). Intelligence and rational behaviour in the bottlenosed dolphin. In: Hurley S. and Nudds M. (eds) *Rational onimols?* Oxford University Press, Oxford. pp. 439-467.

Herman L.M. (2010). What Laboratory Research has Told Us about Dolphin Cognition. *Internotionol Journal of Comparotive Psychology* 23: 310-330.

Herman L.M. (2012). Body and self in dolphins. *Consciousness and Cognition* 12: 526-545.

Herman L.M., Forestell P.H. (1985). Reporting presence or absence of named objects by a language-trained dolphin. *Neuroscience and Biobehovioral Reviews* 9: 667-691.

Herman L.M., Pack A.A., Morrel-Samuels P. (1993). Representational and conceptual skills of dolphins. In: Roitblat H.R., Herman L.M., Nachtigall P. (eds) *Language and Communication: Comporotive Perspectives.* Lawrence Erlbaum, New Jersey. pp. 273-298.

Hunter S., Bay M., Martin M., Hatfield J. (2002). Behavioural effects of environmental enrichment on Harbor Seals (*Phoco vitulina concolor*) and Gray Seals (*Halichoerus grypus*). *Zoo Biology* 21: 375-387.

International Species Information System (ISIS). (2012). Accessed online at http://www.isis.org 4 July 2012.

Jaakkola K. (2012). Cetacean Cognitive Specializations. In: Vonk J. and Shackelford T.K. (eds) *The Oxford Handbook of Comparative Evolutionory Psychology*. Oxford University Press, Oxford. pp. 144-165.

Jaakkola K., Fellner W., Erb L., Rodriguez M., Guarino E. (2005). Understanding of the concept of numerically "less" by bottlenose dolphins (*Tursiops truncatus*). *Journal of Comparative Psychology* 119: 286-303.

Jaakkola K., Guarino E., Rodriguez M., Erb L., Trone M. (2010). What do dolphins (*Tursiops truncatus*) understand about hidden objects? *Animol*

Cognition 13: 103-120.

Joseph B., Antrim J. (2010). Special considerations for the maintenance of marine mammals in captivity. In: Kleiman D.G., Thompson K.V., Baer C.K. (eds) *Wild Mammals in Captivity*. University of Chicago Press, Chicago. pp. 181-196.

Kastelein R.A., Wiepkema PR. (1988). The significance of training for the behaviour of Steller sea lions (*Enmetopias jubata*) in human care. *Aquatic Mammals* 14: 39-41.

Kastelein R.A., Wiepkema P.R. 1989. A digging trough as occupational therapy for Pacific Walruses in human care. *Aquatic Mammals* 15:.9-17.

Kuczaj S.A. II, Lacinak T., Fad O., Trone M., Solangi M., Ramos J. (2002). Keeping environmental enrichment enriching. *International Journal of Comparative Psychology* 15: 127-137.

Kuczaj S.A. II, Lacinak C.T., Turner T.N. (1998). Environmental enrichment for marine mammals at sea world. In: Shepherdson D.J., Mellen J.D., Hutchins M. (eds) *Second nature*: Environmental enrichment for captive animals. Smithsonian Institution Press, Washington DC. pp. 314-328.

Kuczaj S.A. II, Xitco M.J. Jr., Gory J.D. (2010). Can Dolphins Plan their Behavior? *International Journal of Comparative Psychology* 23: 664-670.

Kyngdon D.J., Minot E.O., Stafford K.J. (2003). Behavioural responses of captive common dolphins *Delphinus delphis* to a 'Swim-with-Dolphin' programme. *Applied Animal Behaviour Science* 81: 13-170.

Langbein J., Siebert K., Nürnberg G. (2009). On the use of an automated learning device by group-housed dwarf goats: Do goats seek cognitive challenges? *Applied Animal Behaviour Science* 120: 150-158.

Laule G., Desmond T. (1998). Positive reinforcement training as an enrichment strategy. In Shepherdson D.J., Mellen J.D., Hutchins M. (eds) Second nature: *Environmental enrichment for captive animals*. Smithsonian Institution Press, London. pp. 302-313.

Mackay R.S. (1981) Dolphin interaction with acoustically controlled systems: Aspects of frequency control, learning, and non-food rewards. *Cetology* 41: 1-12.

Marino L., Connor R.C., Ewan Fordyce R., Herman L.M., Hof P.R., Lefebvre L., Lusseau D., McCowan B., Nimchinsky E.A., Pack A.A,. Rendell L., Reidenberg J.S., Reiss D., Uhen M.D., Van de Gucht E., Whitehead H. (2007). Cetaceans have complex brains for complex cognition. *PLoS Biology* 5: 966-972.

Marino L., Frohoff T. (2011). Towards a New Paradigm of Non-Captive Research on Cetacean Cognition. *PLoS ONE* 6: e24121.

Marino L. (2002). Convergence of complex cognitive abilities in cetaceans and primates. *Brain, Behavior and Evolution* 59: 21-32.

Mason G. 1991. Stereotypies: a critical review. *Animal Behaviour* 41: 1015-1037.

Mason G.J., Latham N.R. (2004). Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? *Animal Welfare* 13: 57-69.

Mauck B., Dehnhardt G. (1997). Mental rotation in a California sea lion (*Zalophus californianus*). *Journal of Experimental Biology* 200: 1309-1326.

Meehan C.L., Mench J.A. (2007). The challenge of challenge: Can problem solving opportunities enhance animal welfare? *Applied Animal Behaviour Science* 102: 246-261.

Mercado E. III, Murray S.O., Uyeyama R.K., Pack A.A., Herman L.M. (1998). Memory for recent actions in the bottlenosed dolphin (*Tursiops truncatus):* Repetition of arbitrary behaviors using an abstract rule. *Animal Learning and Behavior* 26: 210-218.

Miller S.J., Mellen J., Greer T., Kuczaj S.A.. (2011). The effects of education programmes on Atlantic bottlenose dolphin (*Tursiops truncatus*) behaviour. *Animal Welfare* 20: 159-172.

Moberg G.P., Mench J.A. (2000). The Biology of Animal Stress. CABI Publishing, New York. pp. 337-354.

Murayama T., Tobayama T. (1995). Mental rotation in beluga (*Delphinapterus leucas*). Technical Report of National Research Institute of Fisheries Engineering. *Fishing Gear and Methods* 6: 9-12.

Pack A.A. (2010). The synergy of laboratory and field studies of dolphin behavior and cognition. *International Journal of Comparative Psychology* 23: 538-565.

Pack A.A., Herman L.M. (2007). The dolphin's (*Tursiops truncatus*) understanding of human gaze and pointing: Knowing what and where. *Journal of Comparative Psychology* 121: 34-45.

Pack A.A., Herman L.M, Hoffmann-Kuhnt M. (2004). Dolphin echolocation shape perception: From sound to object. In: Thomas J.A., Moss C., Vater M. (eds) *Echolocation in Bats and Dolphins*. University of Chicago Press, Chicago. pp. 288-308.

Puppe B., Ernst K., Schön P.C., Manteuffel G. (2007). Cognitive enrichment affects behavioural reactivity in domestic pigs. *Applied Animal Behaviour Science* 105: 75-86.

Ralls K., Fiorelli P., Gish S.. (1985): Vocalizations and vocal mimicry in captive harbour seals, *Phoca vitulina*. *Canadian Journal of Zoology* 63: 1050-1056.

Reiss D., Marino L. (2001). Mirror self-recognition in the bottlenose dolphin: A case of cognitive convergence. *Proceedings of the National Academy of Science* 98: 5937-5942.

Rice D.W. (1998). *Marine mammals of the world: Systematics and distribution*. Society for Marine Mammalogy, Kansas.

Ridgway S., Carder D., Jeffries M., Todd M. (2012). Spontaneous human speech mimicry by a cetacean. *Current Biology* 22: R860-R861.

Rose N.A., Parsons E.C.M., Farinato R. (2009). The case against marine mammals in captivity. The Humane Society of the United States and the World Society for the Protection of Animals.

Ross S.R. (2010). How Cognitive Studies Help Shape Our Obligations for the Ethical Care of Chimpanzees. In: Lonsdorf E.V., Ross S.R., Matsuzawa T. (eds) *The mind of the chimpanzee: Ecological and experimental perspectives.* The University of Chicago Press, Chicago. pp. 309-319.

Samuels A.? Spradlin T. (1995). Quantitative behavioral study of bottlenose dolphins in swim-with-dolphin programs in the United States. *Marine Mammal* Science 11: 520-544.

Savage-Rumbaugh E.S. (1986). Ape language: From conditioned response to symbol. Columbia University Press, New York.

Schusterman R.J., Kastak D. (2004). Problem solving and Memory. In: Hoelzel R (ed) *Marine Mammal Biology: An Evolutionary Approach.* Blackwell, Oxford. pp. 371-387.

Schusterman R.J., Kastak D., Levenson D.H., Reichmuth C.J., Southall B.L. (2000). Why Pinnipeds don't echolocate. *Journal of the Acoustic Society of America* 107: 2256-2264.

Schusterman R.J., Reichmuth Kastak C., Kastak D. (2002). The cognitive sea lion: meaning and memory in the lab and in nature. In: Bekoff M., Allen C., Burghardt G. (eds). *The cognitive animal: empirical and theoretical perspectives on animal cognition*. MIT Press, Cambridge. pp 217-228.

Smith B.P., Litchfield C.A. (2010). An empirical case study examining effectiveness of environmental enrichment in two captive Australian Sea Lions (*Neophoca cinerea*). *Applied Animal Welfare Science* 13: 103-122.

Smolker R.A., Richards A., Connor R., Mann J., Berggren P. (1997). Sponge-carrying by Indian Ocean bottlenose dolphins: possible tool-use by a delphinid. *Ethology* 103: 454-465.

Sobel N., Supin A.Y., Myslobodsky M.S. (1994). Rotational swimming tendencies in the dolphin (*Tursiops truncatus*). *Behavioural brain research* 65: 41-45.

Špinka M., Wemelsfelder F. (2011). Environmental challenge and animal agency. In: Appleby M.C., Mench J.A., Olsson I.A.S. and Hughes B.O. (eds) *Animal Welfare.* CAB International, Wallingford. pp. 27-44.

Sweeney J. (1990). Marine mammal behavioral diagnostics. In: Dierauf L. (ed) *Handbook of Marine Mammal Medicine.* CRC Press, Florida. pp. 53-72.

Thompson R.K.R., Herman L.M. (1977). Memory for lists of sounds by the bottlenosed dolphin: Convergence of memory processes with humans? *Science* 195: 501-503.

Thompson R.K.R., Herman L.M. (1981). Auditory delayed discriminations by the dolphin: Nonequivalence with delayed matching performance. *Animal Learning and Behavior* 9: 9-15.

Visser I.N. (2008). Antarctic peninsula killer whales (*Orcinus orca*) hunt seals and a penguin on floating ice. *Marine Mammal Science* 24: 225-234.

von Fersen L., Schall U., Güntürkün O. (2000). Visual lateralization of pattern discrimination in the bottlenose dolphin (*Tursiops truncatus*). *Behavioural brain* research 107: 177-181.

Xitco M.J., Gory J.D., Kuczaj S.A. (2004). Dolphin pointing is linked to the attentional behavior of a receiver. *Animal Cognition* 7: 231-238.

Xitco M.J., Roitblat HL. (1996). Object recognition through eavesdropping: passive echolocation in bottlenose dolphins. *Animal Learning and Behavior* 24: 355-365.

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Research Article

Feeding practices for captive greater kudus (*Tragelaphus strepsiceros*) in UK collections as compared to diets of free-ranging specimens

Lucy A. Taylor[1,*], Christoph Schwitzer[1], Norman Owen-Smith[2], Michael Kreuzer[3] and Marcus Clauss[4]

[1] *Bristol Conservation and Science Foundation, c/o Bristol Zoo Gardens, Clifton, Bristol BS8 3HA, United Kingdom*
[2] *Centre for African Ecology, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Wits 2050, South Africa*
[3] *Institute of Agricultural Sciences, ETH Zurich, Universitätsstrasse 2, 8092 Zurich, Switzerland*
[4] *Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, 8057 Zurich, Switzerland*
**Corresponding author: ltaylor@bristolzoo.org.uk*

Keywords:
greater kudu, antelope, nutrition, feeding, lucerne hay, crude protein

Article history:
Received: 23 March 2012
Accepted: 25 February 2013
Published online: 24 July 2013

Abstract

Diet-related disorders occur frequently in captive browsing ruminants. To assess current feeding practices, diets of 15 captive greater kudus (*Tragelaphus strepsiceros*) from three zoological collections were compared to that of free-ranging conspecifics and current feeding recommendations. Dry matter intake (DMI) varied considerably in captivity from 49.2 to 75.7 g dry matter kg BM$^{-0.75}$ d^{-1}. Roughage intake determined DMI; with a low DMI associated with low body condition scores (BCS). Compared to free-ranging kudus with diets of 11.1-14.2% crude protein (CP), only one collection had a diet with a similar CP concentration (13.0%), the other collections providing diets with CP of 15.1-16.8%. Acid detergent fibre (ADF) in the zoo diets was comparable to that in the diet of free-ranging animals (24.7-29.4%), but the latter consumed diets with less cellulose and more lignin. In addition, faecal consistency scores (FS) were positively correlated with dietary CP and faecal nitrogen, and negatively with faecal ADF, indicating that higher proportions of highly digestible feeds caused softer faeces. In total, only four of fifteen captive individuals had normal BCS 3.0 and FS 1.0. These results suggest that diets fed to captive browsing ruminants are too digestible, even in terms of fibre composition. Recommendations for higher CP may be unnecessary. Providing browse and high quality lucerne hay may increase the proportion of roughages in the diet and overall DMI to meet requirements and recommendations. To balance the diet and reduce digestibility, the pellets should have lower levels of CP, starch and sugar, and a higher level of fibre, particularly lignin.

Introduction

Browsing ruminants are often considered challenging to maintain in captivity and display a high prevalence of diet-related disorders and poor body condition (Clauss *et al.* 2003; Clauss and Dierenfeld, 2008). In the wild, browsers consume the leaves and twigs of woody plants and forbs, as well as seeds and wild fruits. Generally, browse is higher in crude protein (CP = nitrogen × 6.25) and easily fermentable pectins, and lower in overall fibre, compared to grass (Hummel *et al.* 2006); therefore high CP diets of up to 21% CP were historically recommended for captive browsing ruminants (Fowler 1978; Lintzenich and Ward 1997). However, browse contains more nonproteinacious nitrogen bound to, or in, plant secondary metabolites (PSM) and lignin, which could increase the calculated CP content of browse without increasing available CP; therefore browse probably contains lower levels of available CP than would be expected from its CP concentration (Robbins *et al.* 1987; Hanley *et al.* 1992; Conklin-Brittain *et al.* 1999). In addition, logistical difficulties with providing browse, and the low acceptance of hay in captivity, have led to the relative oversupply of highly digestible feeds to browsing species, such as commercial fruits and cereal-based pellets (Clauss *et al.* 2003). Such diet items are high in sugars and starch, which can cause ruminal acidosis and a number of other diet-related disorders (Potter and Clauss 2005; Clauss and Dierenfeld 2008; Colville *et al.* 2009; Schwitzer *et al.* 2009) and can have serious implications on captive animal health, welfare and longevity and, consequently, conservation strategies for endangered species (Barber 2009; Müller *et al.* 2011). For captive giraffes (*Giraffa camelopardalis*), it is now recommended to reduce the level of CP, sugars and starch, and to increase the content of fibre in the diet (Schmidt and Barbiers 2005; Hummel and Clauss 2006). There is, so far, no reason why these recommendations could not be transferred to other browsing species. Nevertheless, data on the diet selection and nutrient intake of species in the wild is often missing; therefore dietary formulation in captivity is afflicted with a degree of uncertainty (Bond 2001; Wright *et al.* 2011).

Greater kudus (*Tragelaphus strepsiceros*) are browsing ruminants found through much of eastern and southern Africa. The feeding ecology of free-ranging greater kudus has been researched extensively, including research into diet selection (Owen-Smith and Cooper 1987; Owen-Smith 1994; de Garine-Wichatitsky *et al.* 2004; Hooimeijer *et al.* 2005; Codron *et al.* 2007a) and estimations of nutrient and energy intake (Owen-Smith and Cooper 1989; Owen-Smith 1997). Comparing the diet of captive greater kudus to their free-ranging counterparts could highlight existing problems in captive feeding practices to help reduce the putative incidence of diet-related disorders. Therefore, the aim of this study was to assess the current feeding practices for captive greater kudus by comparing the dietary and faecal nutrient composition between captive and free-ranging greater kudus and the current feeding recommendations for captive browsing ruminants.

Methods

Captive greater kudus

The research was conducted following the methods described for captive tapirs (Clauss *et al.* 2009). Fifteen greater kudus from three zoological collections in the United Kingdom were assessed during November 2010. The body mass (BM) of each animal was estimated by keeper and researcher assessment, together with a photographic comparison to free-ranging greater kudus, and to the other greater kudus in this study. Each animal was assigned a faecal consistency score (FS) calculated from the mean score of all observed defecations of the respective individual (Table 1; Figure 1), and a body condition score (BCS) (Table 2).

FS	Description
1.0	Firm, individual pellets; cylindrical in shape with a point at one end (~15-20mm long)
1.5	Softer rounder pellets
2.0	Soft pellets, larger in size and beginning to stick together
3.0	Firm faecal mass. All pellets clumped together, with a range of pellet sizes.
4.0	Soft faecal mass, with little form.
5.0	Fluid

Table 1. Faecal consistency score (FS) chart for captive greater kudu developed for this study, with the aid of Greeff and Karlsson (1997) and Clauss et al. (2009). Normal free-ranging greater kudu faeces (Stuart and Stuart, 2000) would be FS 1.0 using this chart.

Food intake was measured over a consecutive 3-day period by weighing individual food items offered and subtracting the mass of the food not consumed on a daily basis. At zoo A, meadow hay was available in a mixed species exhibit; thus hay intake was estimated by observations and by weighing representative samples of hay that replicated the observed amounts of hay ingested by kudus. The animals at zoos B and C had access to grass pasture; grass ingestion could not be measured in this study. In all zoos, samples were taken of pelleted feeds, forages, browse and faeces. Pellet and hay samples were stored in paper bags. Browse and faeces were stored in sealed plastic bags and frozen.

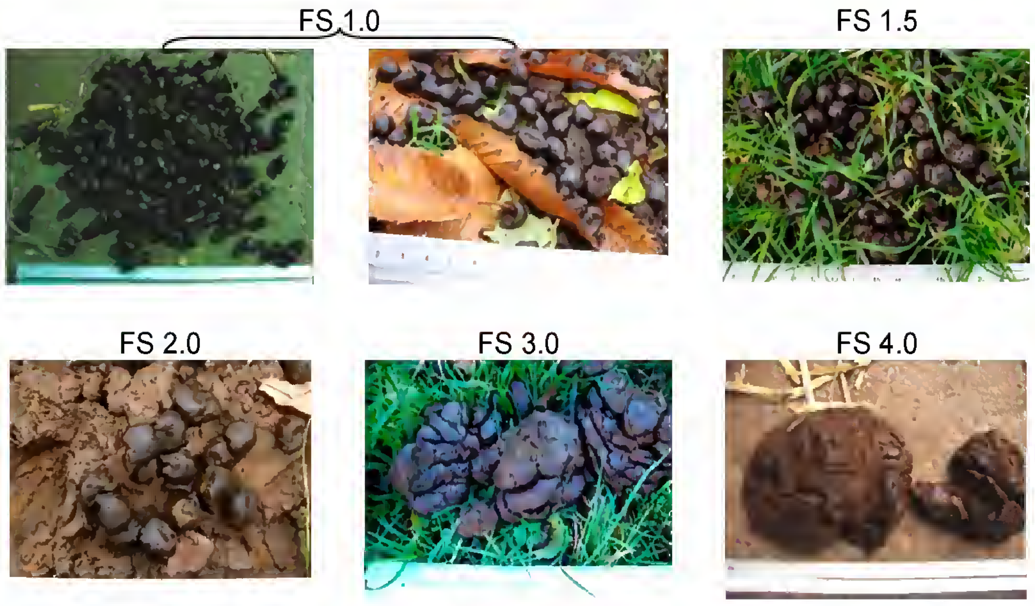


Figure 1. *Examples of faecal consistency scores for captive greater kudu.*

Both feed and faecal samples were dried at 60°C for 48 h and then milled in a Retsch mill with a 0.5 mm sieve (Retsch, Haan, Germany). Dry matter (DM) and total ash were determined with a TGA-500 furnace (Leco Corporation, St. Joseph, MI, USA; AOAC, 1997, no. 942.05) heated at steps of 105°C and 550°C, respectively. The content of CP was determined as nitrogen × 6.25 on a C/N analyzer (Leco-Analysator Typ FP-2000, Leco Instrumente GmbH, Kirchheim, Germany) (AOAC no. 977.02). Neutral detergent fibre (NDF, with addition of α-amylase and without sodium sulfite), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined following Van Soest *et al.* (1991) by using the Fibertec apparatus (Fibertec System M, Tecator, 1020 Hot Extraction, Flawil, Switzerland). All fibre values are expressed without residual ash. As in Clauss *et al.* (2009), fat content was estimated, on a DM basis, as 4% in browse, 2% in hay, and, for manufactured feeds, the fat value was obtained from the feed packaging. Nutrient contents of fruits and vegetables were obtained from ZOOTRITION™ software (version 2.6, Saint Louis Zoo, MO, USA).

Cellulose was calculated as ADF-ADL, non-ADF-carbohydrates (NadfC% DM) were calculated as 100-CP-fat-ash-ADF; and non-fibre carbohydrates (NFC% DM), where fibre is equal to NDF, were calculated as 100-CP-fat-ash-NDF. The metaboliable energy (ME) content of the diet was estimated from the nutrient composition using calculations by Pellew (1984) for estimating the energy intake of free-ranging giraffe as follows:

$$\text{Gross energy (GE MJ/kg DM)} = 0.0226\ \text{CP (g/kg DM)} + 0.0407\ \text{fat (g/kg DM)} + 0.0192\ \text{ADF (g/kg DM)} + 0.0177\ \text{NadfC (g/kg DM)}$$

$$\text{Metabolizable energy (ME MJ/kg DM)} = 0.447 \times \text{GE}.$$

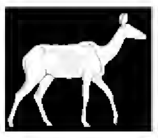
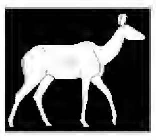
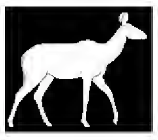
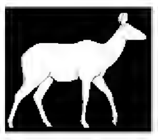
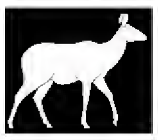
BCS	Diagram (Female)	General	Neck/ shoulder	Ribs	Back	Hip/ tailhead
1.0		Emaciated, skeletal	Emaciated bone structure of shoulders and very thin neck	Prominent and easily visible	Very prominent ridge, spinous processes are identifiable	Pelvic bones are very prominent
2.0		Thin	Thin neck and shoulders	Visible	Prominent ridge	Pelvis bones may be slightly discernable, but hips are rounded
3.0		Good, fit	Moderate covering	Not visible	No ridge	Fat is present around the tailhead, pelvic bones are flat
4.0		Fat	Thick neck, shoulders slightly rounded	Not visible, fat deposits may be present	Back appears flatter, fat deposits present	Hips are rounded
5.0		Obese	Thick neck, rounded shoulders, fat deposits	Not visible, fat deposits may be evident	Wide, flat back with patchy fat deposits	Hips are very round

Table 2. Body condition score (BCS) chart for captive greater kudu developed for this study, with the aid of Riney (1960), Clauss et al. (2009) and Disney's Animal Kingdom (2005). Normal BCS is 3.0.

The proportional consumption of food, nutrients and ME by each individual in the group was presented on the basis of the calculated metabolic body mass (BM, $kg^{0.75}$).

Free-ranging greater kudus

The BSC and FS of free-ranging greater kudus were assumed to be normal for this species at 3.0 and 1.0, respectively. Dietary nutrient concentrations were estimated from data collected by Owen-Smith and Cooper (1989), who followed four free-ranging subadult greater kudus for one year and recorded the food intake of three different individuals, from dawn to dusk, on three days each month. For each plant consumed, the time spent foraging was multiplied by the eating rate (g DM per minute, from the product of bite size and biting rate) to estimate the DM intake of each plant. The dietary concentrations of CP, cellulose and ADL in DM were then calculated from the chemical composition of each plant species. The results were split into a mean for the dry season, using the data from June until September, and the wet season from November until April, according to the seasonal notes presented in Owen-Smith and Cooper (1989). For faecal N, ADF and ADL, the data from Codron *et al.* (2007b) was used as comparison. Cellulose was calculated as described above.

Zoo diet evaluation

The zoo diets were evaluated by comparing the dietary and faecal nutrient concentrations to those of free-ranging greater kudus, and to the feeding recommendations of Lintzenich and Ward (1997; recalculated to a DM basis), for medium to large browsers, and Schmidt and Barbiers (2005) and Hummel and Clauss (2006), for captive giraffes. Lucerne hay quality was assessed according to the quality standards of the American Forage and Grassland Council, from high "prime quality" hay, followed by "quality 1" hay down to low "quality 4" hay, using CP and NDF proportions in the DM of the lucerne hay (Baylor and Rohweder 1979). The ME intake of the captive greater kudus was compared to the estimated maintenance ME requirements of captive mammals, calculated using the allometric equation for the basal metabolic rate of 0.293 MJ kg $BM^{-0.75}$ day^{-1}, multiplied by factors of 1.5 and 2.0, to give a maintenance requirement of 0.44-0.59 MJ ME kg $BM^{-0.75}$ d^{-1} (Kirkwood 1996). Spearman's rank correlation coefficient was used to test for a correlation between the dietary and faecal nutrient concentrations, and between BCS or FS and the dietary and/or faecal nutrients, using IBM® SPSS® Statistics 19 software (SPSS Inc. Chicago, IL). Associations between BCS and the intake of nutrients (g kg $BM^{-0.75}$ day^{-1}) were tested by correlation analysis, because overall intake affects BCS; similarly, associations between FS and the concentrations of nutrients in DM were tested for, because diet composition affects FS. The significance level was set to $p<0.05$.

Results

The mean BCS at zoo A was 3.0±0.0 (mean ± standard deviation), at zoo B 2.7±0.4 and at zoo C 3.3±0.3. Eight of 15 animals scored normal BCS 3.0, with three animals at zoo B below, and four animals at zoo C above, this value (Table 3). The mean FS at zoo A was 1.04±0.06, at zoo B 2.0±1.1 and at zoo C 2.5±1.1. One animal could not be assessed for FS at zoo B. Only one of 14 individuals assessed for FS achieved a mean FS of 1.0. Four individuals, including both animals at zoo A and two non-lactating females at zoo B, scored BCS 3.0 and had a mean FS lower than 1.5.

Dry matter intake (DMI), excluding grass, ranged from 49.2 g DM kg $BM^{-0.75}$ d^{-1} at zoo B to 75.7 g DM kg $BM^{-0.75}$ d^{-1} at zoo A (Table 4). All collections provided pelleted feed and lucerne hay at *ad libitum* access. The DMI from pellets was relatively consistent across all collections. When two types of pellets were offered, the pellets were mixed in a pre-defined ratio. No selection bias was observed between pellets during this study.

Zoo	Animal	Type	BM (kg)	BCS	FS
A	1	Adult female	180	3.0	1.08
	2	Adult female	170	3.0	1.00
B	1	Adult female	170	3.0	1.25
	2	Adult female	150	2.5	2.67
	3	Adult female	150	3.0	-*
	4	Adult female	150	3.0	1.25
	5	Lactating female	160	2.0	4.00
	6	Subadult male	100	3.0	1.50
	7	Juvenile	40	2.5	1.50
C	1	Adult male	230	3.0	2.50
	2	Adult female	190	3.5	1.75
	3	Lactating female	190	3.0	4.25
	4	Lactating female	180	3.5	3.25
	5	Juvenile	60	3.5	1.25
	6	Juvenile	50	3.5	1.75

*Faeces could not be accurately identified, but appeared to be FS 1.5

Table 3. Group composition at each zoological collection, including body mass (BM), body condition score (BCS) and faecal score (FS) of each individual. Normal BCS is 3.0 and normal FS is 1.0.

Zoo	DMI in order of Inclusion (*excluding grass*)	Total DMI	Fruits/ Veg		Pellets			Roughage			Browse			Grass	Unstr : struct ratio
			Access	% Diet	Access	% Diet	DMI	Access	% Diet	DMI	Access	% Diet	DMI	Access	
A	Browser breeder pellets [a]; Lucerne hay; Meadow hay; Acacia pellets [b]; Hazel (*Corylus avellana*); Stinging Nettles (*Urtica dioica*); Maple (*Acer* sp.); Willow (*Salix* sp.); Broccoli; Apples; Cauliflower; Carrots	75.7	Limited	1.7	Limited, but leftovers	48.6	36.8	*Ad lib.*	38.5	29.1	Limited	11.2	8.5	None	1 : 1
B	Ungulate rolls [c]; Lucerne hay; Molassed dried alfalfa	49.2	None	0.0	Limited	68.8	33.9	Dried Alfalfa: limited; Lucerne hay: *Ad lib.*	31.2	15.4	When available; not during trial	0.0	0.0	24 hours	2.2 : 1
C	Browser cubes [d]; Lucerne hay; Linseed lozenges [e]; Pear; Carrots; Apples; Grapes	63.2	Limited	5.8	*Ad lib.*	51.7	32.7	*Ad lib.*	42.5	26.8	When available; not during trial	0.0	0.0	~8 hours	1.4: 1

Main ingredients in order of inclusion:

[a] Browser breeder pellets: Oat hulls and bran, soya bean hulls, dehulled extracted toasted soya, lucerne meal, molasses, wheat, dextrose monohydrate;

[b] Acacia pellets: *Acacia* sp., Hominy chop, molasses meal, yellow maize, lucerne, soya oilcake, cotton oilcake, sunflower oilcake;

[c] Ungulate rolls: Barley, wheatfeed, wheat, molaferm 20 mollases bulk, calcium cabonate, oat pellets, sunflower ext, blended vegetable oil;

[d] Browser cubes: Grass, unmolassed sugar beet, wheat, dehulled soya bean meal, soya hulls, oatfeed, full fat linseed;

[e] Linseed lozenges: Linseed expeller, glucose syrup.

Table 4. Dietary composition, excluding grass, of the captive greater kudus at each collection in terms of dry matter intake (DMI = g DM kg $BM^{-0.75}$ d^{-1}). Unstructured food includes fruit/ vegetables and pellets and structured food includes roughage and browse.

None of the collections applied a ratio of 30-40% pellets to 60-70% roughage and browse, as recommended by Lintzenich and Ward (1997), but zoo A just attained the recommendations of Hummel and Clauss (2006), of a maximum of 50% unstructured food (fruit/veg and pellets), at 50.4%. The DMI of lucerne hay increased as hay quality increased, but none of the collections fed “prime quality” lucerne hay as recommended by Lintzenich and Ward (1997) (Figure 2). The intake of meadow hay at zoo A, which was in a daytime mixed species exhibit, was affected by the dominance of the other species in the exhibit. With the inclusion of meadow hay and medium quality lucerne hay, the animals at zoo A had a higher roughage intake than the animals at zoo C with high quality lucerne hay (29.1 compared to 26.8 g DM kg BM$^{-0.75}$ d^{-1}). In addition, zoo A was the only collection that provided seasonal browse on a daily basis, which increased the total DMI and meant zoo A had an overall roughage and browse intake of 37.6 g compared to the lowest roughage intake of 15.4 g DM kg BM$^{-0.75}$ d^{-1} at zoo B. Commercial fruits and vegetables were fed on a daily basis to the animals at zoo C; at zoo A, apples and carrots were only fed as part of the twice-weekly training program, and broccoli and cauliflower were fed as enrichment 2-3 times a week, alternating with stinging nettles (*Urtica dioica*); no fruits or vegetables were fed at zoo B. The animals with access to grass pasture at zoos B and C were observed grazing, but grass consumption could not be quantified.

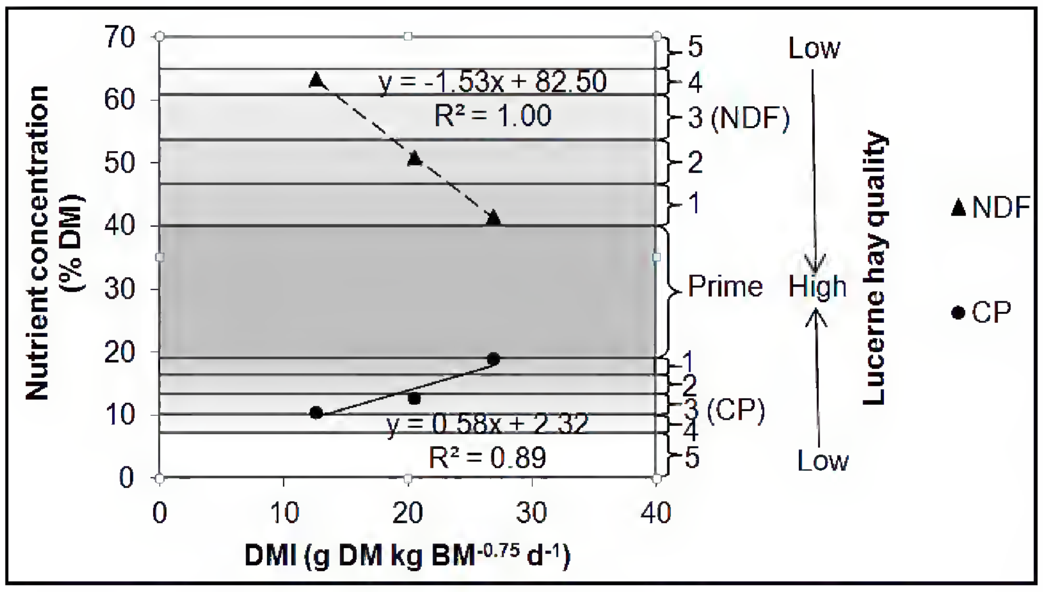


Figure 2. Relationship between lucerne hay dry matter intake (DMI = g DM kg BM$^{-0.75}$ d^{-1}) and quality. Lucerne hay quality is assessed according to the quality standards of the American Forage and Grassland Council, using the crude protein (CP% DM) and neutral detergent fibre (NDF% DM) content (Baylor and Rohweder 1979). Prime quality is highest quality hay.

Dietary CP concentration at zoo A was comparable to both the free-ranging greater kudus and the recommendations of Schmidt and Barbiers (2005) of 10-14% CP (Table 5). Zoo C was within the range of the higher recommendations of Lintzenich and Ward (1997) of 16.7-21.1% CP. The lowest intake of CP was at zoo B, despite a dietary CP concentration of 15.1%, due to the low total DMI. The dietary ADF concentrations at zoos A and C and of the free-ranging greater kudus (24.7-29.4%) were comparable to the levels recommended for captive giraffes by Schmidt and Barbiers (2005) of 25-30% ADF. However, free-ranging greater kudus consumed a ratio of cellulose to ADL of 1.6-1.7, whereas captive greater kudus consumed more cellulose and less ADL in a ratio of 2.4 at zoo B and 2.9 at both zoos A and C (Table 6). In terms of lucerne hay, the ratio of cellulose to ADL ranged from 3.8 in medium-low quality lucerne hay to 3.0 in high “quality 1” lucerne hay, which was still high compared to 0.3-1.9 in the browse at zoo A. The highest percentage of NFC (% DM) in the diet was at zoo B, due to high levels in the pelleted feed (49.7%). In the faecal analysis, free-ranging greater kudus had higher faecal N and ADL concentrations than captive greater kudus.

In terms of ME intake, zoo C was the only collection within the estimated maintenance ME requirements of 0.44-0.59MJ kg BM$^{-0.75}$ d^{-1}. Zoo A provided over (105-140%), and zoo B provided under (67-90%), the estimated ME requirement, respectively.

There were significant, positive correlations between dietary CP and faecal N (rs = 0.55; p = 0.044), and dietary and faecal NDF (rs = 0.83, p<0.001) and cellulose (rs = 0.55, p = 0.029) concentration, but there was a negative correlation between dietary and faecal ADL concentration (rs = -0.61, p = 0.020). BCS was significantly positively correlated with DMI (g DM kg BM$^{-0.75}$ d^{-1}) (rs = 0.54, p = 0.038). However, BCS only showed a tendency to correlate with energy intake (rs = 0.49, p = 0.061) and was not associated with FS (rs = -0.13, p = 0.650). The FS significantly increased with dietary CP concentration (% DM) (rs = 0.58, p = 0.030), but was not significantly correlated with other dietary nutrients. In the faecal analysis, there was a highly significant, negative correlation between FS and faecal DM content (rs = -0.82, p <0.001), suggesting that firmer faeces were also less moist. FS was also positively correlated with faecal N concentration (% DM) (rs = 0.65, p = 0.013) and negatively correlated with faecal ADF concentration (rs = -0.64, p = 0.014) (Figure 3).

Zoo	Mean BCS	Mean FS	Crude protein		NDF (% DM)	ADF (% DM)	ADL (% DM)	NFC (% DM)	Metabolizable energy	
			% DM	g kg BM$^{-0.75}$ d^{-1}					MJ/kg DM	MJ kg BM$^{-0.75}$ d^{-1}
A	3.0	1.0	13.0	9.8	44.3	30.5	7.9	32.3	8.14	0.62
B	2.7	2.0	15.1	7.4	33.1	22.9	6.7	40.5	7.99	0.39
C	3.3	2.5	16.8	10.6	35.7	25.4	6.9	33.8	8.07	0.51

Table 5. The body condition score (BCS), faecal score (FS) and dietary nutrient concentration (% DM) and intake (g kg BM$^{-0.75}$ d^{-1}) (neutral detergent fibre – NDF, acid detergent fibre – ADF, acid detergent lignin – ADL; non-fibre carbohydrates - NFC) of the captive greater kudus in this study. Normal BCS is 3.0 and normal FS is 1.0 and metabolizable energy is estimated.

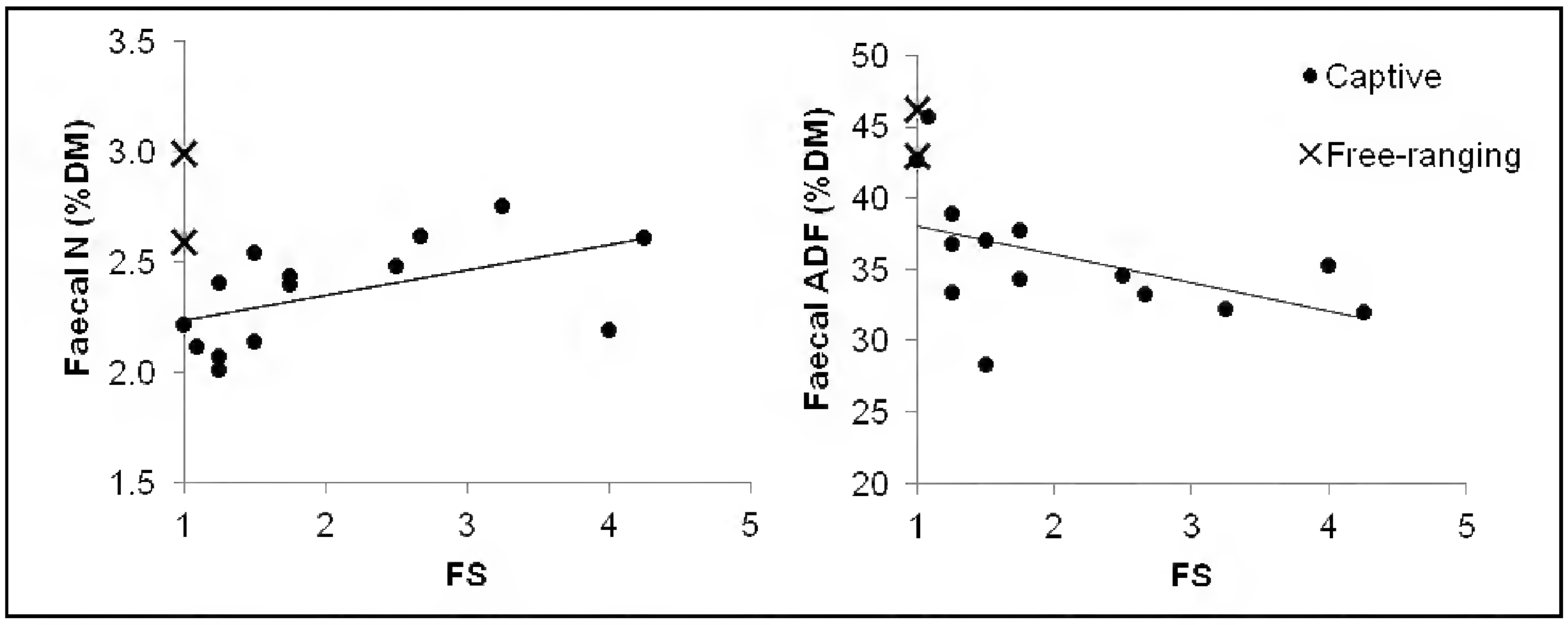


Figure 3. Relationship between the mean faecal consistency score (FS) and (a) faecal nitrogen (N) and (b) faecal acid detergent fibre (ADF) of captive and free-ranging greater kudus. Normal FS is 1.0.

Dataset		Dietary CP (% DM)	Faecal N (% DM)	Cellulose (% DM)		ADL (% DM)		Ratio of cellulose to ADL	
				Diet	Faeces	Diet	Faeces	Diet	Faeces
Captive greater kudus	Zoo A	13.0	2.3	22.6	29.3	7.9	14.9	2.9	2.0
	Zoo B	15.1	2.2	16.2	15.0	6.7	19.3	2.4	0.8
	Zoo C	16.8	2.5	18.5	19.2	6.9	15.4	2.9	1.2
Free-ranging greater kudus*	Dry season	11.1	2.6	15.8	19.8	13.6	26.5	1.7	0.8
	Wet season	14.2	3.0	13.7	19.9	11.1	23.1	1.6	0.6

* From the dataset of Owen-Smith and Cooper (1989) and the data of Codron et al. (2007)

Table 6. Dietary and faecal concentrations of crude protein (CP) or nitrogen (N), cellulose and in captive and free-ranging greater kudus

Discussion

The results of the present study suggest that captive greater kudus were less likely to achieve a normal BCS, and tended towards softer faeces, than their free-ranging counterparts, which suggests that the diets in captivity differed in nutrient composition from the diet in the wild. Some of the limitations of the study must be stated. This study was on a small scale involving 15 animals on three different diets. The body mass of each individual was estimated and no adjustments were made for the requirements of lactating or growing individuals; thus the maintenance levels presented represent a minimum group requirement. The dietary and faecal nutrient concentrations were measured over three days and do not encompass long-term consequences or seasonal variations. For logistical reasons, the intake of grass and the individual feed intake of each animal could not be measured. The dietary and faecal nutrient concentrations in free-ranging greater kudus were obtained from two different sources (c.f. Owen-Smith and Cooper 1989; Codron *et al.* 2007b). Nevertheless, the findings facilitate the identification of major differences between diets consumed in captivity and in the wild.

The BCS was rather low in three animals of zoo B, which suggests a deficient intake of energy or specific nutrients or both. The positive correlation between DMI and BCS, and an ME intake below the estimated minimal requirements, suggests that the animals at zoo B did not consume enough food to meet requirements. Assuming an ME content of 8 MJ/kg DM, a non-reproducing adult would have to consume 55-74 g DM kg BM$^{-0.75}$ d^{-1} to meet the estimated ME requirements of 0.44-0.59 MJ kg BM$^{-0.75}$ d^{-1}. A DMI below these estimations, like at zoo B at 49.2 g DM kg BM$^{-0.75}$ d^{-1}, suggests the presence of either an energy deficit, or a nutrient-dense ration that could, in turn, lead to diet-related disorders, such as acidosis. However, there is also an inverse relationship between both DMI and ME intake and access to grass. This correlation could suggest that animals with greater grass access, such as the animals at zoo B, ate more grass, to the extent that grass DMI depressed DMI (excluding grass). However, grass is generally high in cellulose and abrasive silica. Browsers, with 'moose-type anatomy', achieve lower fibre digestive coefficients than grazers (Pérez-Barberìa *et al.* 2004) and may not be adapted to comminute grass effectively (Hummel *et al.* 2008). This is reflected in the diet of free-ranging greater kudus, which only consists of approximately 4% tropical grass (Codron *et al.* 2007a). Although four animals at zoo B did achieve a normal BCS, three animals, including a lactating female, had a lower BCS, which suggests that the animals may not have been able to extract sufficient nutrients from the grass to maintain an animal with additional requirements. Therefore, pasture grass should not be considered a major component in the diet of captive greater kudus, and access to pasture should not imply that a lower provision of roughages, such as lucerne hay or browse, is required. The animals at zoo A were consuming more than the estimated maximum maintenance ME requirements, but given that both animals scored BCS 3.0, the long term balance and digestion of nutrients appears to have met requirements. However, it should be noted that ME was only estimated in this study. The DMI from the pellets was relatively consistent across all collections, despite differences in the type and availability of pelleted feed. Instead, DMI was affected by the intake of roughages and browse, which varied considerably. Therefore, to increase the DMI (for example at zoo B), the intake of roughages must be increased.

Roughage intake is important for the stimulation of rumination and normal feeding behaviour. Yet, there are numerous reports documenting the low acceptance of hay by captive browsing ruminants (c.f. Clauss *et al.* 2003). Miller *et al.* (2010) reported a low intake of timothy hay by captive greater kudus, with a pellet to timothy hay ratio of 5.0:1.0. By comparison, the animals at zoo C had an unstructured to structured food ratio of 1.4:1.0, using high “quality 1” lucerne hay. Lucerne hay DMI appeared to be directly related to quality as, even with a limited quantity of alternative feeds and the prospect of energy deficiency, the animals at zoo B, on low “quality 4” lucerne hay, consumed much less lucerne hay than the other animals in this study with *ad libitum* access to pellets. High-quality lucerne hay has also been reported to increase roughage intake in captive giraffes and okapis (*Okapi johnstoni*) (Hummel *et al.* 2003), and high-quality forage is generally associated with higher food intake in herbivores (Meyer *et al.* 2010). However, prime quality lucerne hay can be difficult to source in Europe (Clauss and Dierenfeld 2008). Wright *et al.* (2011) reported significant variation in the quality of lucerne hay fed to eastern bongos (*Tragelaphus eurycerus isaaci*) in UK collections. The provision of browse, as shown in the present study and by Hatt *et al.* (2005) in captive giraffe, can increase DMI and should be offered as much as possible. However, obtaining browse in large quantities can be logistically difficult, and expensive. Ideally, zoos that keep browsing ungulates should maintain browse plantations (Höllerl *et al.* 2006; Schlegel *et al.* 2006). By providing browse, and both lucerne and grass hay, zoo A attained the highest roughage intake, with an unstructured to structured food ratio of 1.0:1.0. A diverse diet has been shown to increase DMI in several domestic species (Rolls *et al.* 1983; DiBattista and Sitzer 1994; Villalba *et al.* 2011). Therefore, providing a greater variety of roughages, including grass hay, may increase roughage and total DM intake. Increasing the number of different roughages offered could also positively impact on animal welfare because a monotonous diet might cause satiety, which might limit food intake and could be stressful for the animal (Villalba *et al.* 2010). Low-quality lucerne hay of grades 3-4 (c.f. Ullrey 1997) should be avoided due to the low intake by the captive greater kudus.

In this study, FS was positively correlated to both dietary CP and faecal N concentration (% DM). Nijboer *et al.* (2006) also reported a positive correlation between dietary and faecal CP concentration and FS in captive colobine monkeys (Subfamily: Colobinae), where the proportion of CP ingested by captive individuals was higher than the CP consumed by their free-ranging counterparts. Indeed, compared to the free-ranging greater kudus, the diets of the captive greater kudus with a higher FS had a much higher proportion of CP.

In cattle (*Bos primigenius*), a high CP concentration can cause softer FS, but FS is also affected by CP digestibility (Ireland-Perry and Stallings 1993). Whether it is really the high CP concentration (and digestibility) as such, or whether CP is only a general proxy in these cases for overall diet quality, remains to be elucidated.

The higher faecal N in captive animals, with a higher FS, is an indirect indication of a highly digestible diet (Hodgman *et al.* 1996). Low fibre diets are also associated with softer FS in a number of different species (Ireland-Perry and Stallings 1993; Nijboer *et al.* 2006). In the present study, there was a negative correlation between dietary and faecal indigestible ADL concentration across the three collections, which confirms that diets lower in ADL, or fibre as a whole, also had a higher digestibility. Diets that were lower in fibre and roughages contained a higher proportion of pelleted feed and either a cereal-based pellet or commercial fruits, which are known to be highly digestible (Clauss and Dierenfeld 2008). Correspondingly, there was a negative correlation between FS and faecal ADF concentration, which suggests that a higher content of highly digestible feeds, with a lower content of fibre, contributed to, or caused, the high FSs in the present study. Therefore, the present results support the notion that highly digestible feeds should not be fed in large amounts to captive browsing ruminants, and that the content of CP, sugars and starch should be reduced by increasing the content of fibre, ideally by the proportion of roughages, in the diet of captive browsing ruminants.

The recommendations of Lintzenich and Ward (1997), of 16.7-21.1% CP, exceed the CP concentration of the diet actually consumed by free-ranging subadult greater kudus. These diets contained 11.1% CP in the dry season and 14.2% CP in the wet season. This could be an indication that current protein recommendations are unnecessarily high. McCusker *et al.* (2011) found that growing mule deer (*Odocoileus hemionus*) were able to meet both energy and protein requirements on a low-starch high-fibre pellet with 14.8% CP. In addition, the higher faecal N concentration of free-ranging greater kudus, with a normal FS, could be the result of a higher content of indigestible N bound to, or in, plant secondary metabolites or lignin, which could be an indication of different N to CP conversion factors (Robbins *et al.* 1987; Hanley *et al.* 1992; Conklin-Brittain *et al.* 1999). Therefore, the actual dietary CP available for digestion to the free-ranging greater kudus may be comparatively lower than that in captive animals consuming a diet with the same nominal CP concentration. In the present study, the non-reproducing greater kudus at zoo A were maintained with a FS lower than 1.5, and at a BCS of 3.0, with a dietary CP concentration of 13.0%, which suggests that the reduced protein recommendations for captive giraffes by Schmidt and Barbiers (2005) of 10-14% CP could be applied to captive greater kudus as well. Further research is required into CP digestibility, and into N conversion factors for wild and zoo diets.

The proportion of unstructured feeds at two collections was above the maximum of 50% as recommended by Hummel and Clauss (2006). Apart from contributing to, or causing, softer FSs, more seriously, diets high in sugars and starch and low in fibre and roughages can cause ruminal acidosis, oral stereotypies and other diet-related disorders (Schochat *et al.* 1997; Schaub *et al.* 2004; Potter and Clauss 2005; Clauss and Dierenfeld 2008; Colville *et al.* 2009). Therefore, cereal-based pellets and commercial fruits should be removed from the diet, or restricted to behavioural training, and the proportion of fibre and roughages in the diet should be increased. The proportion of ADF in the diet of free-ranging greater kudus was comparable to the recommendations for giraffe of Schmidt and Barbiers (2005) of 25-30%, which again suggests that these recommendations could be implemented for captive greater kudus. However, free-ranging greater kudus had a higher proportion of indigestible ADL in the diet, which suggests that the current diets fed to captive browsing ruminants are too digestible, even in terms of fibre composition. High-quality lucerne hay contains a lower proportion of cellulose, and has a lower cellulose to ADL ratio, than low-quality lucerne hay, but still has a relatively high cellulose to ADL ratio (3.0) compared to the diet of free-ranging greater kudus (1.6-1.7). Feeding of lucerne hay is recommended not for its protein content, but because it is a readily available roughage that will ensure fibre intake. Providing browse, high-quality lucerne hay and a pelleted feed with a high fibre content and a low cellulose to ADL ratio appear to be the best available recommendations for the feeding of captive greater kudu based on the present findings.

Conclusions

1. In a study of the current feeding practices for captive greater kudus, only eight of 15 individuals had a normal body condition score and five individuals had a normal faecal consistency score, which suggests that the amount and the composition of the diets fed to captive greater kudus were different from that ingested by their free-ranging counterparts.
2. A low body condition score was correlated with a low intake of total dry matter, confirming the usefulness of condition scoring to monitor nutritional status and dietary adequacy.
3. Faecal consistency was positively correlated with dietary CP and faecal N and negatively correlated with faecal ADF, which suggests that a higher content of highly digestible feeds may have contributed, or caused, softer faeces and should therefore not be fed to captive greater kudus.
4. Compared to free-ranging greater kudus, captive greater kudus consumed diets with a much higher concentration of CP, which appears to be unnecessary, and with a lower cellulose to ADL ratio, indicating that the fibre composition in diets in captivity deviates from conditions in the wild.
5. Roughage intake had the greatest effect on DMI in captivity. However, lucerne hay intake increased as hay quality increased. Hence, rather than trying to meet energy and nutrient requirements by offering a nutrient dense diet with some roughage, the findings of this study suggest that feeding browse, high-quality low fibre roughage and high fibre, low CP, starch and sugar, pellets, is a better strategy.
6. It should be further tested whether the feeding recommendations for captive giraffes, of 10-14% CP and 25-30% ADF by Schmidt and Barbiers (2005), could also be implemented for captive greater kudus.

Acknowledgements

We thank the BIAZA Research Committee for their support with this research project and the zoological collections and their keepers for their help with this study. We also thank Dr Stephen Waite, Ailison Kelland and Rebecca Sealy at Hartpury College for providing equipment and storing samples. LT would also like to thank the Hardy Family and Zoo Zürich for their hospitality during data collection and analysis, respectively, and John and Fiona Taylor for help with sample storage and transportation.

References

AOAC (1997) *Official methods of analysis*. Association of official analytical chemists, Arlington, VA, USA.

Barber J.C. (2009) Programmatic approaches to assessing and improving animal welfare in zoos and aquariums. *Zoo Biology* 28:519-530.

Baylor J.E., Rohweder D.E. (1979) Implementation of new hay standards in the U.S. --- Where are we? *Report from the AFGC Hay Marget Task Force. Proc Ann AFGC* pp. 47-57. Littlerock, AK.

Bond A. (2001) Practical problems with data collection for nutritional analysis: a study of animal diets at Bristol Zoo Gardens. In: Fidgett A.L., Clauss M., Eulenberger K., Hatt J.-M., Hume I., Janssens G., Nijboer J., editors. *Zoo Animal Nutrition.* vol III. Fürth, Germany: Filander Verlag.

Clauss M., Dierenfeld E.S. (2008) The nutrition of "browsers". In: Fowler M.E., Miller R.E., editors. *Zoo and Wild Animal Medicine: Current Therapy*. vol 6. St. Louis, USA: Saunders Elsevier. p 444-454.

Clauss M., Kienzle E., Hatt J.-M. (2003) Feeding practice in captive wild ruminants: peculiarities in the nutrition of browsers/ concentrate selectors and intermediate feeders. A review. In: Fidgett A.L., Clauss M., Gansloßer U., Hatt J.-M., Nijboer J., editors. *Zoo Animal Nutrition*. vol II. Fürth, Germany: Filander Verlag. p 27-52.

Clauss M., Wilkins T., Hartley A., Hatt J.-M. (2009) Diet composition, food intake, body condition, and fecal consistency in captive tapirs (*Tapirus* spp.) in UK collections. *Zoo Biology* 28:279-291.

Codron D., Codron J., Lee-Thorp J.A., Sponheimer M., de Ruiter D., Sealy J., Grant R., Fourie N. (2007a) Diets of savanna ungulates from stable carbon isotope composition of faeces. *Journal of Zoology* 273:21-29.

Codron D., Lee-Thorp J.A., Sponheimer M., Codron J., De Ruiter D., Brink J.S. (2007b) Significance of diet type and diet quality for ecological diversity of African ungulates. *The Journal of Animal Ecology* 76:526-537.

Colville K., Bouts T., Hartley A., Clauss M., Routh A. (2009) Frothy bloat and serous fat atrophy associated with insufficient fibre intake in a giraffe (*Giraffa camelopardalis*). In: Clauss M., Fidgett A.L., Hatt J.-M., Huisman T., Hummel J., Janssen G., Nijboer J., Plowman A., editors. *Zoo Animal Nutrition*. Fürth, Germany: Filander Verlag. p 219-229.

Conklin-Brittain N.L., Dierenfeld E.S., Wrangham R.W., Norconk M., Silver S.C. (1999) Chemical protein analysis: A comparison of Kjeldahl crude protein and total ninhydrin protein from wild, tropical vegetation. *Journal of Chemical Ecology* 25:2601-2622.

de Garine-Wichatitsky M., Fritz H., Gordon I.J., Illius A.W. (2004) Bush selection along foraging pathways by sympatric impala and greater kudu. *Oecologia* 141:66-75.

DiBattista D., Sitzer C.A. (1994) Dietary variety enhances meal size in golden hamsters. *Physiology & Behavior* 55:381-383.

Disney's Animal Kingdom (2005) *Nyala body condition scores*. Lake Buena Vista, Florida, USA: Disney's Animal Kingdom.

Fowler M.E. (1978) Peracute mortality in captive giraffe. *Journal of the American Veterinary Medical Association* 173:1088-1093.

Greeff J.C., Karlsson L.J.E. (1997) Genetic relationships between faecal worm egg count and scouring in merino sheep in a mediterranean environment. *Proceedings of the Association of the Advancement Animal Breeding and Genetics* 12:333-337.

Hanley T.A., Robbins C.T., Hagerman A.E., McArthur C. (1992) Predicting digestible protein and digestible dry matter in tannin-containing forages consumed by ruminants. *Ecology* 73:537-541.

Hatt J.-M., Schaub D., Wanner M., Wettstein H.R., Flach E.J., Tack C., Hässig M., Ortmann S., Hummel J., Clauss M. (2005) Energy and fibre intake in a group of captive giraffe (*Giraffa camelopardalis*) offered increasing amounts of browse. *Journal of Veterinary Medicine* 52:485-490.

Hodgman T.P., Davitt B.B., Nelson J.R. (1996) Monitoring mule deer diet quality and intake with fecal indices. *Journal of Range Management* 49:215-222.

Höllerl S., Stimm B., Hummel J., Clauss M. (2006) Browse provision for captive herbivores: design and management of a browse plantation. In: Fidgett A.L., Clauss M., Eulenberger K., Hatt J.-M., Hume I., Janssens G., Nijboer J., editors. *Zoo Animal Nutrition*. vol III. Fürth, Germany: Filander Verlag.

Hooimeijer J.F., Jansen F.A., de Boer W.F., Wessels D., van der Waal C., de Jong C.B., Otto N.D., Knoop L. (2005) The diet of kudus in a mopane dominated area, South Africa. *Koedoe* 48:93-102.

Hummel J., Clauss M. (2006) Feeding. In: Barta Z., Clauss M., Culik L., Damen M., Hummel J., Schleussner G., Tomasova K., Zimmermann W., editors. *EAZA husbandry and management guidelines for Giraffa camelopardalis*. Arnhem: Burger's Zoo. p 26-61.

Hummel J., Fritz J., Kienzle E., Medici E.P., Lang S., Zimmermann W., Streich W.J., Clauss M. (2008) Differences in fecal particle size between free-ranging and captive individuals of two browser species. *Zoo Biology* 27:70-77.

Hummel J., Hoerhager A., Nawrocki D. (2003) Selective browsers - adequate nutrition of giraffes and okapis in zoos. *Z Kölner Zoo* 46:67-80.

Hummel J., Südekum K.-H., Streich W.J., Clauss M. (2006) Forage fermentation patterns and their implications for herbivore ingesta retention times. *Functional Ecology* 20:989-1002.

Ireland-Perry R.L., Stallings C.C. (1993) Fecal consistency as related to dietary composition in lactating holstein cows. Journal *of Dairy Science* 76:1074-1082.

Kirkwood J.K. (1996) Nutrition of captive and free-living wild animals. In: Kelly N., Wills J., editors. *BSAVA Mannual of Small Animal Nutrition*. London: The British Veterinary Association. p 235-243.

Lintzenich B.A., Ward A.M. (1997) Hay and pellet ratios: considerations in feeding ungulates. *Nutrition Advisory Group Handbook Fact Sheet 006.*

McCusker S., Shipley L.A., Tollefson T.N., Griffin M., Koutsos E.A. (2011) Effects of starch and fibre in pelleted diets on nutritional status of mule deer (*Odocoileus hemionus*) fawns. *Journal of Animal Physiology and Animal Nutrition* 95:489-498.

Meyer K., Hummel J., Clauss M. (2010) The relationship between forage cell wall content and voluntary food intake in mammalian herbivores. *Mammal Review* 40:221-245.

Miller M., Weber M., Valdes E.V., Neiffer D., Fontenot D., Fleming G., Setter M. (2010) Changes in serum calcium, phosphorus, and magnesium levels in captive ruminants affected by diet manipulation. *Journal of Zoo and Wildlife Medicine* 41:404-408.

Müller D.W.H., Bingaman Lackey L., Streich W.J., Fickel J., Hatt J.-M., Clauss M. (2011) Mating system, feeding type and ex situ conservation effort determine life expectancy in captive ruminants. *Proceedings of the Royal Society* B 278:2076-2080.

Nijboer J., Clauss M., Everts H., Beynen A. (2006) Effect of dietary fibre on the faeces score in colobine monkeys in Dutch zoos. In: Fidgett A., Clauss M., Eulenberger K., Hatt J.-M., Hume I., Janssens G., Nijboer J., editors. *Zoo Animal Nutrition*. vol III. Fürth: Filander Verlag. p 145-155.

Owen-Smith N. (1994) Foraging responses of kudus to seasonal changes in food resources: elasticity in constraints. *Ecology* 75:1050-1062.

Owen-Smith N. (1997) Control of energy balance by a wild ungulate, the kudu (*Tragelaphus strepsiceros*), through adaptive foraging behaviour. *Proceedings of the Nutritional Society* 56:15-24.

Owen-Smith N., Cooper S.M. (1987) Palatability of woody plants to browsing ruminants in a South African savanna. *Ecology* 68:319-331.

Owen-Smith N., Cooper S.M. (1989) Nutritional ecology of a browsing ruminant, the kudu (*Tragelaphus strepsiceros*), through the seasonal cycle. *Journal of Zoology* 219:29-43.

Pellew R.A. (1984) The feeding ecology of a selective browser, the giraffe (*Giraffa camelopardalis*). *Journal of Zoology* 202:57-81.

Pérez-Barberia F.J., Elston D.A., Gordon I.J., Illius A.W. (2004) The evolution of phylogenetic differences in the efficiency of digestion in ruminants. *Proceedings of the Royal Society B* 271:1081-1090

Potter J.S., Clauss M. (2005) Mortality of captive giraffe (*Giraffa camelopardalis*) associated with serous fat atrophy - a review of 5 cases at Auckland zoo. *Journal of Zoo and Wildlife Medicine* 36:301-307.

Riney T. (1960) A field technique for assessing physical condition of some ungulates. *Journal of Wildlife Management* 24:92-94.

Robbins C.T., Hanley T.A., Hagerman A.E., Hjeljord O., Baker D.L., Schwartz C.C., Mautz W.W. (1987) Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68:98-107.

Rolls B.J., Van Duivenvoorde P.M., Rowe E.A. (1983) Variety in the diet enhances intake in a meal and contributes to the development of obesity in the rat. Physiology & Behavior 31:21-27.

Schaub D., Clauss M., Flach E.J., Wettstein H.R., Tack C., Hatt J.-M. (2004) Influence of physical and chemical composition of diet on oral stereotypies in captive giraffes (*Giraffa camelopardalis*). *Proceedings of the European Association of Zoo and Wildlife Veterinarians* (EAZWV). Ebeltoft, Denmark. p 27-28.

Schlegel M.L., Renjifo A., Valdes E.V. (2006) Nutrient content of Carolina willow (*Salix caroliniana*) browse components fed to exotic ruminants. In: Fidgett A., Clauss M., Eulenberger K., Hatt J.-M., Hume I., Janssens G., Nijboer J., editors. *Zoo Animal Nutrition*. vol III. Fürth, Germany: Filander Verlag. p 213-224.

Schmidt D.A., Barbiers R. *The giraffe nutrition workshop proceedings*; 2005; Lincoln Park Zoo, Chicago, Illinois.

Schochat E., Robbins C.T., Parish S.M., Young P.B., Stephenson T.R., Tamayo A. (1997) Nutritional investigations and management of captive moose. *Zoo Biology* 16:479-494.

Schwitzer C., Polowinsky S.Y., Solman C. (2009) Fruit as foods - common misconceptions about frugivory. In: Clauss M., Fidgett A., Hatt J.-M., Huisman T., Hummel J., Janssen G., Nijboer J., Plowman A., editors. *Zoo Animal Nutrition*. vol IV. Fürth, Germany: Filander Verlag.

Stuart C., Stuart T. (2000) *A field guide to the tracks and signs of southern and East African wildlife*. 3rd ed. Cape Town: Struik Publishers.

Ullrey D.E. (1997) Hay quality evaluation. *Nutrition Advisory Group Handbook Fact Sheet 001.*

Van Soest P.J., Robertson J.B., Lewis B.A. (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74:3583-3597.

Villalba J.J., Bach A., Ipharraguerre I.R. (2011) Feeding behavior and performance of lambs are influenced by flavor diversity. *Journal of Animal* Science 89:2571-2581.

Villalba J.J., Provenza F.D., Manteca X. (2010) Links between ruminants' food preference and their welfare. *Animal* 4:1240-1247.

Wright D.J., Omed H.M., Bishop C.M., Fidgett A.L. (2011) Variations in Eastern bongo (*Tragelaphus eurycerus isaaci*) feeding practices in UK zoological collections. *Zoo Biology* 30:149-164.

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Research Article

Evaluating the activity patterns and enclosure usage of a little-studied zoo species, the sitatunga (*Tragelaphus spekii*)

Paul Rose[1,2,*] and Ryan Robert[1]

[1]*H.E. Animal Management, Sparsholt College Hampshire, Winchester, SO21 2NF, United Kingdom*
[2]*Centre for Research in Animal Behaviour, College of Life & Environmental Sciences, University of Exeter, Exeter, EX4 4QT, United Kingdom*
**Corresponding author: paul.rose@sparsholt.ac.uk*

Keywords:
Sitatunga, behaviour, activity budget, enclosure evaluation, evidence-based husbandry.

Article history:
Received: 22 February 2012
Accepted: 13 July 2012
Published online: 24 July 2013

Abstract

Ungulates can be underrepresented in zoo animal behaviour and welfare research, yet they comprise some of the most widely-kept captive species and as such, their lives within the zoo are worthy of closer investigation. Sitatunga (*Tragelaphus spekii)* are kept in numerous zoological collections globally yet current information on species-specific husbandry requirements and captive behaviour patterns appears limited. Many enclosures for ungulates can be uninspiring and generic; this study was designed to gain a better understanding of daily activity and enclosure use of a species that, in the wild, has a very particular habitat choice. Data were collected at the former Cricket St Thomas Wildlife Park, Chard, UK on eight sitatunga to determine overall daily activity patterns and usage of all available areas of their exhibit. Instantaneous scan sampling of the whole herd during three periods each day (morning, midday and afternoon) allowed for changes in behaviour patterns to be assessed over time. The enclosure encompassed both biologically-relevant (long grasses, reeds and shallow water) and less relevant (open, short-grassed) areas; these were zoned according to features considered useable to the sitatunga and that could influence behaviour and time spent within that zone. Zone usage was analysed using a modified Spread of Participation Index (SPI) which indicated a significant preference for biologically-relevant spaces. Significantly enhanced behavioural repertoires occurred in the "natural" zones of the enclosure and three behaviours (standing, sitting/ruminating and eating) showed significant differences in performance between natural and artificial zones, and between time of day. Captive sitatunga display a daily rhythm in their activity, however comparison with wild data in the literature shows only few similarities in daytime activity budget and analysis reveals a significant difference between daily feeding patterns. Overall, enclosure design based on facets of natural ecology is important for the expression of a "wild-type" behaviour pattern in captive ungulates and sitatunga will actively choose more biologically-relevant areas of their exhibit when these are available. It is suggested that alterations to husbandry regime and management style of such specialised ungulates could help improve captive behavioural repertoires and enhance the display of such animals in the zoo.

Introduction

When designing zoo animal enclosures it is important to have knowledge of the activity budgets of the species intended for exhibition to determine optimal furnishing and size allocation, thus providing quality space for the animal (Estevez and Christman 2006; Ross *et al.* 2009). Simple methods of space-use evaluation and assessment have been applied to several commonly held zoo taxa (e.g. Hedeen 1983; Hebert and Bard 2000; Mallapur *et al.* 2002; Ross and Lukas 2006; Ross *et al.* 2011) and it is understood that the design of enclosures (and hence the environment created for a species) plays an important role in determining the overall diversity of activity budgets shown by captive species (Reinhardt *et al.* 1996; Seidensticker and Forthman 1998; Clark *et al.* 2012; Rose and Roffe 2012). Species-appropriate exhibitory that facilitates the performance of self-fulfilling behaviours (e.g. appetitive behaviours, as per Day *et al.* 1995; Duncan 1998) is enriching to the animal and may ultimately provide a better quality experience for the zoo visitor (Blowers *et al.* 2012; Fabregas *et al.* 2012).

It is well-known that measurement of such "useable space" can be undertaken via a calculation of Spread of Participation Index (SPI), either in its traditional or modified form (see Hedeen 1983; Plowman 2003). SPI results enable those providing care, designing enclosures and manipulating social groupings to have quantifiable data on which to base decisions pertinent to individual species. Evaluation of captive provision is required for species commonly kept but perhaps overlooked; animals that may not outwardly show signs of stress but who may benefit from an assessment of husbandry, management and provision within their collection. Such an evidence-based approach (Melfi 2009) is a useful direction for zoos to travel in to make substantial and long-term changes to husbandry and enclosure design. This evidence-based style has been the subject of the research project presented here.

The sitatunga (*Tragelaphus spekii*) is an example of a species held in captivity at a range of institutions (being kept in 78 animal collections throughout the world, see ISIS as of February 2012) but where little research into captive need has been conducted. Wild sitatunga face a current downward population trend although they are not currently classed as threatened on the IUCN Red List (IUCN SSC Antelope Specialist Group 2008). A semi-aquatic antelope occupying marshes and swamps, papyrus beds and the wetter areas of savannah and rainforest habitats (Kingdon 1982; Estes 1991; MacDonald 2001; Robinchaud 2011), the wild activity budget of the sitatunga can be difficult to define, posing a challenge to those seeking the "evidence-basis" for zoo husbandry.

Information presented in Delany and Happold (1979) and Kingdon (1982) states that animals prefer to browse in low-growing thicket in and around marshy areas, as well as entering deeper water to consume vegetation. Owen (1970) details foraging patterns with sitatunga consuming a wide variety of wetland plants, and again, Kingdon's (1982) review of sitatunga grazing behaviour in wetland areas details animals regularly leaving resting areas of dense cover to forage on new-growth grass. Consequently, for a meaningful exhibit to be constructed in the zoo, key aspects of evolutionary biology, ecological niche and behavioural ecology must be incorporated. The minimum husbandry guidelines developed by the Association of Zoos and Aquaria (AZA) do provide basic husbandry needs for the keeping of Tragelaphine antelopes (Antelope TAG 2006) but no specialist requirements are stated for individual species. These AZA guidelines, together with some detail on the World Association of Zoo and Aquariums (WAZA) website, are only minimum standards and zoos are expected to expand on them to incorporate biologically-important features within species-specific exhibits.

Data presented here will hopefully enable those managing sitatunga populations to have a greater understanding of captive needs and show an example of evidence-based animal management whereby changes can be implemented from a sound understanding of need and requirement. Whilst complete re-creation of the biome so favoured by this antelope (Plate 1) may not always be totally feasible, key facets of this biome could be included to remove the generalised look of an exhibit (e.g. Plate 2).

Plates 1 (left) and 2 (right). A replication of the sitatunga's natural habitat in the American Museum of Natural History, and a familiar, less diverse, habitat provided for the same species in captivity (author's photographs, 2011).

Data were collected to answer the hypotheses a) sitatunga will preferentially favour specific areas of an exhibit that best mimic wild-type conditions and that b) time budgets / behavioural repertoires of sitatunga in the zoo will mirror those of wild, free-living animals.

Methods

Behavioural data were collected at Cricket St Thomas Wildlife Park, Chard, UK in July 2009 for ten consecutive days. The study location was chosen due to the perceived quality of the sitatunga paddock, which contained a range of different "habitats" for the antelope to use and showed environmental heterogeneity not always that evident for captive ungulates. Observations were made in three time periods (09:00-11:00, 12:00-13:00 and 14:00-16:00 BST) using instantaneous scan sampling with an interval time of two minutes. A total of 50 hours of data was gathered on each individual antelope. These three observation periods were selected to best replicate the division of daytime activity that would be seen in the wild (and hence to allow for differences in behaviour at varying time periods to be recorded) and to work around the husbandry regime at the collection at that time. Animals were individually identifiable via different coloured ear tags so that, with the aid of binoculars, identification was always possible. The sitatungas' paddock was divided up into zones (Table 1) with some areas containing features that replicated biologically-relevant habitats and others that were deemed less ecologically important. These zones were designated and mapped using a satellite picture of the paddock obtained from Google Earth^{TM} Pro with the areas (m^2) of each section being calculated. At the same time as behavioural sampling, location of each animal within the exhibit was. recorded

Zone	Name	Area (m^2)
1	Back grassed area	3754
2	End waterfall	102
3	Deep pool	311
4	Pool with trees	271
5	Centre waterfall	153
6	Shallow pool	682
7	Bank with reeds and grass	1181
8	Start waterfall	46
9	Front grassed area	3403
10	Housing	166
11	Marshy area	243
Total area of enclosure = 10312m^2		

Table 1. Designated zones with the sitatunga enclosure and the area of each.

Behaviour		Description
	Standing	The animal is upright on all four limbs, not moving, with its torso raised off ground, eyes open and observant of the immediate environment.
Grouped into sit / ruminate for analysis.	**Sitting**	The animals is conscious and in a ventral recumbent position on the ground.
	Rumination	Jaw movements associated with mastication of food or a bolus for the process of digestion of plant material. Normally performed sitting down, quietly, in a sheltered area of the enclosure.
Grouped into feeding for analysis.	**Feeding and foraging**	The action of eating or consuming forage, browse or grass using the tongue, teeth and mouth. The animal can be upright or sitting.
	Foraging	The animal is actively seeking and searching for food using its senses to search out food. The animal is moving purposefully around the exhibit sniffing, tasting and exploring different foodstuffs.
Grouped into other state behaviour for analysis.	**Sleeping**	The animal is in a complete or partial state of unconsciousness; in a dormant state, with eyes closed, resting on the ground.
	Aggression	Any antagonistic interaction between or directed to other individuals; this can include clash fighting where horns are used against another animal, chasing of one animal by another in a threatening manner, pushing, shoving or displacement of one individual by another.
	Walking	The animal is travelling on foot, advancing the feet alternately so that there is always two or more feet on the ground at any one time, either individually or as a herd.
	Scratching	The animal uses horns, mouth or limbs to manipulate or rub a part of its body in a short, sharp rhythmic pattern.
	Play	The animal engages in physical activity that is undertaken purely for enjoyment or amusement; this can include skipping, "gambolling", running, chasing or cavorting with other individuals in the enclosure. There is no associated aggressive response. In male animals only, sparring ("play fighting") may be performed between two individuals.
	Vigilance	The animal, whilst standing, assumes an alert posture and stares fixedly on a specific point with both ears forward; the animal may produce an alarm snort or stamp the ground with one leg.
	Territorial actions	A directed social interaction of one individual towards another to denote possession of a specific area of ground. Once the targeted animal moves away, the interaction ceases. In a male animal only, the antelope may "horn" the ground or associated enclosure furnishings.

Table 2. Ethogram of captive sitatunga behaviour observed in the study

The eight sitatunga (two adult males, five adult females, one juvenile of unknown gender) were all ear-tagged. The two male antelope, one older than the other, could be identified by their physical size and horn length. All five females were identifiable by the position and colour of their ear tags. The juvenile sitatunga was identifiable due to its size in comparison to other members of the study group.

Behavioural recording, undertaken by the same sole researcher for the duration of the observation period, took place from a small hide placed in the enclosure in a position that provided an uninterrupted view of all animals in all areas of the enclosure. A seven day pilot study and acclimatisation period were instigated before the main data collection period commenced. Using Popp (1982) and Estes (1991) as a main guide, an ethogram of key behaviours was created to categorise activity observed (Table 2). To enable clarity in analysis of the results, observed behaviours were grouped into four main categories, which also facilitated the comparison of sitatunga behaviour with that in published work.

Data were analysed using Microsoft Excel© 2008 and Minitab© Statistical Software, version 16. Evaluation of zone usage was undertaken using the Modified SPI formula (Plowman 2003). A value of 0.0 suggests equal use of all zones whereas a value of 1.0 suggests use of only one zone, with the formula for calculation of zone use being: $SPI = (S \mid f_o - f_e \mid) / [2 (N - f_e min)]$ whereby f_o is the observed frequency in each zone, f_e is the expected frequency for each zone and f_e min the expected frequency in the smallest zone.

Behavioural data were normally distributed and a two-way ANOVA was used to determine differences between wild and captive time budgets, and for analysing differences between the animals' behaviour at different sample points. Analysis of enclosure zone use (non-parametric data) was conducted with one-factor Chi-squared tests. Wild/captive activity comparisons were analysed using tabulated behavioural data published by Owens (1970) from a group of free-living sitatunga.

Results

Analysis of time spent by each animal in the different zones showed an overall preference to spend time in the more biologically-relevant areas of the exhibit. In total, 27.5% of the enclosure falls into biologically-relevant areas (zones 3, 4, 5, 6, 7 and 11) and 54% of the total study time showed animals to be in these zones. A one-factor Chi-squared analysis confirmed a significant difference between zone uses for the group as a whole; $\chi^2_{[1]}=86.9$, $p< 0.005$.

All sitatunga appeared to favour zone 7, the "river bank" area with long grass and reeds (Table 3). The male animals also spent more time in the house than the females. The juvenile spent more time out on the short grass areas than the rest of the herd. No animals were seen to use zone 8, the waterfall at the start of the exhibit. Figure 1 shows the total time (as a % of the overall observation period) that each individual spent in the biologically-relevant zones.

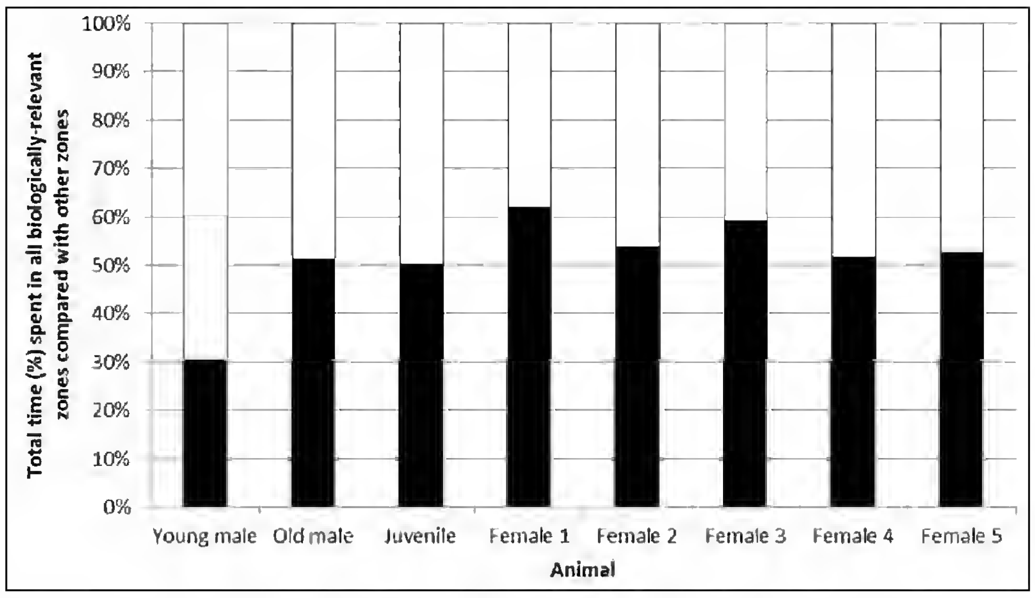

Figure 1. Total time spent by individual sitatunga at Cricket St Thomas Wildlife Park in two main classes of zone in the enclosure. Black = all biologically-relevant zones; white = all other zones.

It would be interesting to investigate further any social or hierarchical influences that were reducing the time the young male was able to spend in areas that were preferentially frequented by the other antelope. A modified SPI analysis for each demographic provides the following results; for the adult male sitatunga SPI = 0.64; for the young male = 0.74; and for female sitatunga SPI = 0.59. For the group as a whole, an SPI value of 0.61 was calculated for zone usage for the entire observation period. A one factor Chi-squared analysis was also used to analyse the number of behaviours performed in either category of zone and this showed there to be a significant difference in the total number of behaviours recorded between the different zones ($\chi^2_{[1]} = 104.11$, $p<0.05$). Similar analyses were conducted for three key behaviours (standing, sitting and feeding), and sitting and eating behaviours were found to be performed for significantly different amounts of time between natural and artificial zones ($\chi^2_{[1]} = 609.64$, $p<0.05$; $\chi^2_{[1]} = 143.18$, $p<0.05$) respectively.

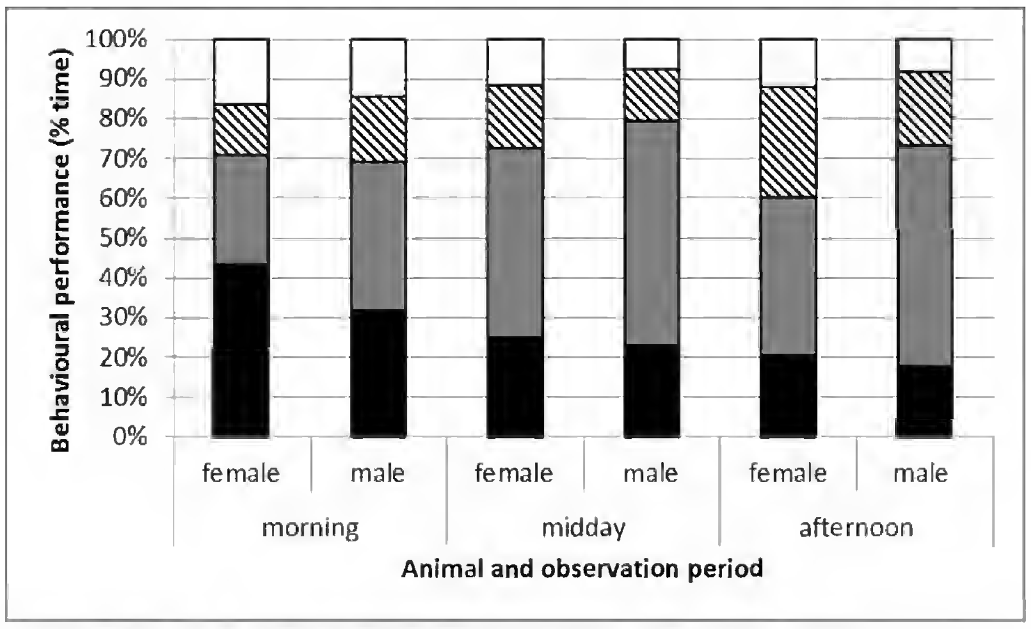

Figure 2. Daily activity pattern of both female and male sitatunga over an average day of the study period. Black =standing; grey = sitting / ruminating; dashed black = feeding; white = other state behaviour. Sitting has been grouped with ruminating as this the common position that this behaviour was observed in.

	Biologically-relevant Zones					Other Zones					
	Zone 3	**Zone 4**	**Zone 5**	**Zone 6**	**Zone 7**	**Zone 11**	**Zone 1**	**Zone 2**	**Zone 8**	**Zone 9**	**Zone 10**
Young male	0	10	4	38	860	10	24	0	0	298	1776
Old male	8	4	2	42	1482	10	4	224	0	272	972
Juvenile	24	94	0	120	1270	6	74	0	0	1228	204
Female 1	28	16	22	138	1662	0	146	134	0	508	366
Female 2	18	8	0	72	1522	0	32	306	0	232	830
Female 3	22	4	4	80	1678	2	40	48	0	358	784
Female 4	0	8	56	142	1360	0	284	0	0	336	834
Female 5	0	118	4	180	1256	30	102	10	0	780	540

Table 3. Time (in minutes) each individual sitatunga spent in each zone of the enclosure for the total duration of the study. See Table 1 for a description of each zone.

Figure 2 shows fluidity in behaviour across the observation period for the group of sitatunga, as well any differences in behavioural performance between the sexes. Inferential analysis shows there to be a significant difference between gender and time spent sitting ($F_{[1]}=182.28$, $p=0.005$) as well as for time of day when animals were observed sat down ($F_{[2]}=474.04$, $p=0.002$). Likewise, the same differences but analysing when animals chose to feed, also showed a significant relationship ($F_{[1]}=18.30$, $p=0.051$ and $F_{[2]}=49.61$, $p=0.02$).

Figure 3 provides an interesting comparison between wild and captive sitatunga behaviour as it shows only limited similarity in the activity budget of both populations, especially concerning time spent performing "other" behaviours in the morning and at midday. Deviations away from the natural activity pattern of the wild individuals are seen in this captive group concerning performance of key appetitive behaviours such as feeding and sitting/ruminating. Wild animals stand more in the middle of the day and feed more in the morning.

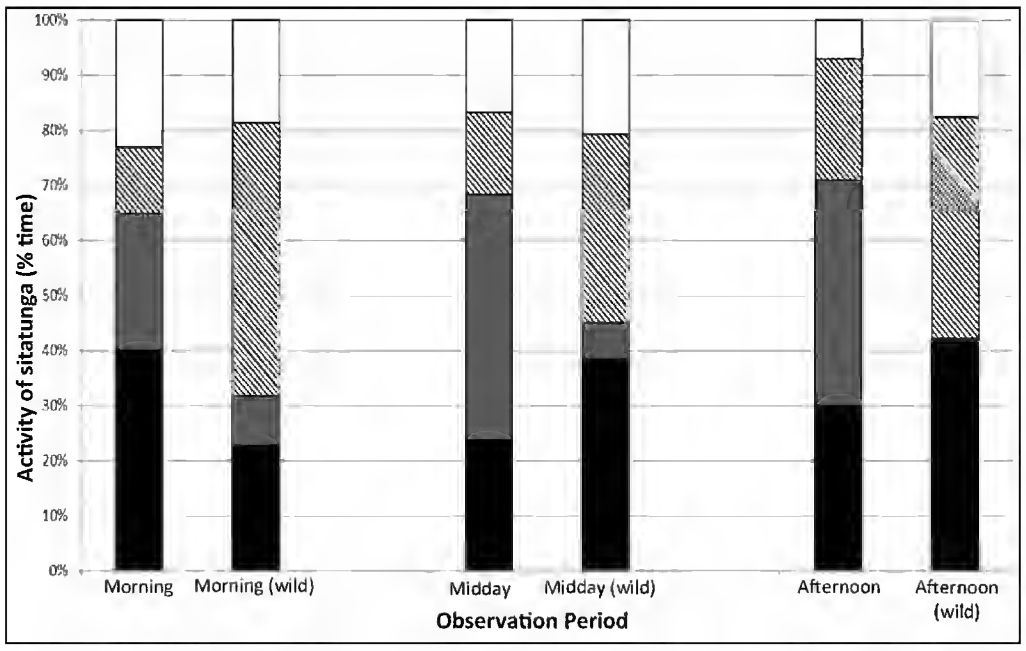

Figure 3. Overall group time budget for the whole observation period, split into the three specific sampling times. Black =standing; grey = sitting / ruminating; dashed black = feeding; white = other state behaviour. Wild data adapted from that published in Owens (1970), page 186, table 3. Data used is from time periods that compared to those used for the captive study. Sitting is compared to Owens' category of "lying" and other (for both captive and wild) includes other normal state behaviours (e.g. grooming, locomotion).

Wild sitatunga also display 1% of their time lying / sitting / ruminating in the afternoon whereas this behaviour comprises a substantial proportion of the activity budget of the captive animals (41%). Captive sitatunga show a less defined rhythm to their feeding activities, which is perhaps to be expected in an artificially managed environment. A two-way ANOVA shows there to be a significant difference for time spent feeding between each population at each time of day ($F_{[1]}=29.56$, $p=0.032$).

Numerous authors expand on the sitatunga's preference for long grass and reedy habitats. The biologically-relevant areas of the enclosure included only one section (zone 7) of long grasses/reeds; 11% of the total space available to the sitatunga. Figure 4 shows the time each sitatunga spent this zone; there is no significant difference between individuals in time spent in zone 7 ($\chi^2_{[7]}= 7.57$; $p<0.05$), so it could assumed to be equally important to all animals in this herd. The importance of "natural" zones for performance of behaviours with a strong motivation is illustrated by Figure 5.

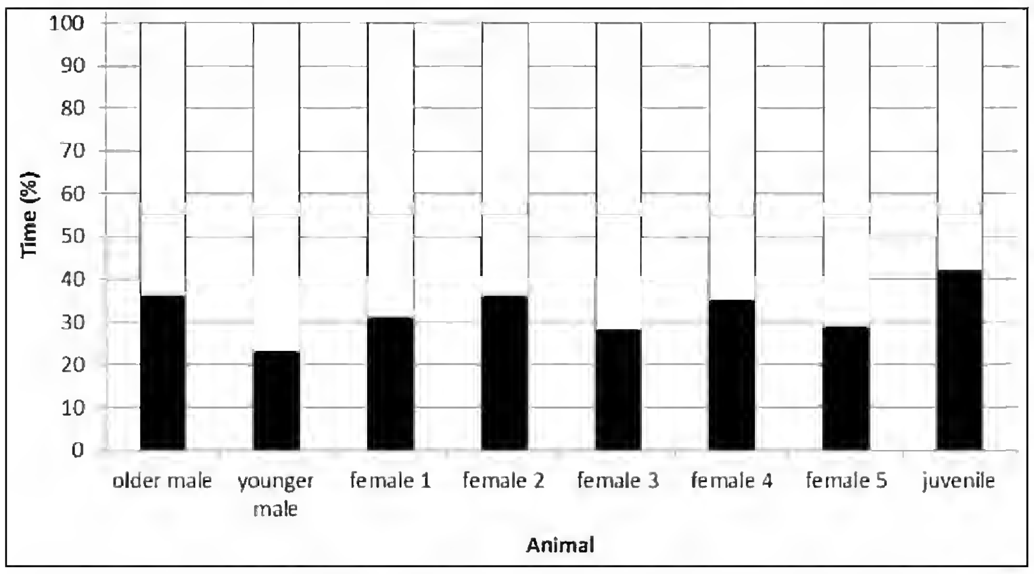

Figure 4. Comparison of time spent in a biologically-important area, long grass and reeds (black section) compared to time spent in the rest of the enclosure (white section).

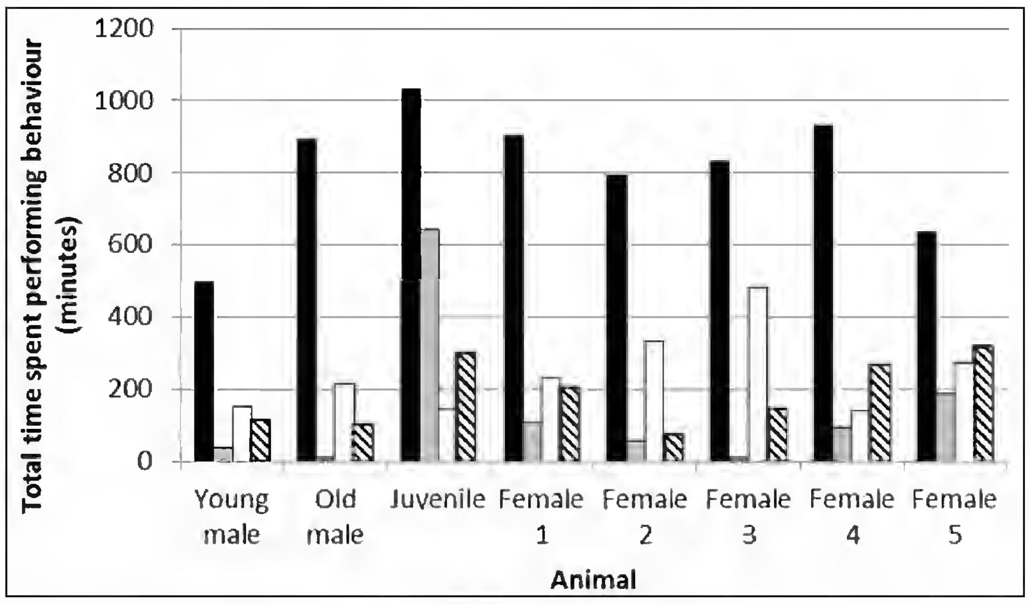

Figure 5. Performance of key appetitive behaviours by individual sitatunga in biologically-relevant and other enclosure zones. Black = sitting/ruminating (biologically-relevant zones); Grey = feeding/foraging (biologically-relevant zones); White = sitting/ruminating (other zones); Dashed line = feeding/foraging (other zones).

Discussion

The results show that various aspects of the sitatungas' behaviour are significantly different between biologically-relevant and other enclosure zones. The frequencies of three behaviours (standing, sitting/ruminating and feeding) were significantly different from morning to afternoon, thereby reflecting, in part, natural activity patterns. The modified SPI value (0.61) indicates that the sitatunga were not utilising all aspects of their enclosure equally and the sitatungas' significant preference for reedy / long-grassy areas is supported by the inferential analysis and by Figure 5. The herd spent 54% of its time in only 28% of the enclosure, suggesting that improvements to the remaining 72% encourage more widespread movement and a fuller development of a daily activity pattern.

The increased usage of these natural areas may also be explained by the behavioural ecology of the sitatunga and the need to conduct important state behaviours (feeding, resting, standing) in an environment most suitable for their performance. Comparing with observations from Owen (1970), Kingdon (1982), Games (1983), Estes (1991), Starin (2000) and Robinchaud (2011), sitatunga were observed to remain sedentary in reedy, or long-grass beds during the day; such habitats were reflected in this captive setting and the same behaviours were observed within them. Important functions of long-grass for sitatunga in the wild include places to hide from predators, provision of shelter from high temperature and sites for rumination (Estes 1991; Wronski *et al.* 2006). Figure 4 shows the relative proportion of time spent in a relatively small area of the enclosure (11%) compared to all of the other space available to the animals. Whilst not all behavioural functions are required per se in a captive setting, it is important to provide animals with the choice to perform behaviour that has a high motivational value (Duncan 1998); such activities can be reliable indicators of positive welfare (de Jonge et al. 2008). It is recommended that sitatunga be given more areas of long grass and reeds to facilitate their apparent preference for this type of "habitat".

None of the sitatunga in this study displayed any form of stereotypy, such as repetitive oral movements or pacing (Bashaw *et al.* 2001; Baxter and Plowman, 2001). As stated by Mason and Mendl (1997) many stereotypical behaviour patterns are linked to an inability to perform appetitive foraging behaviours. Sitatunga are both grazers and browsers, and as a result spend long periods of time foraging for specific plant materiel (Estes 1991; Skinner and Chimimba 2006). As no stereotypies were displayed it can be deduced that the area of, and resources within, the natural zones were currently sufficient to prevent the need for the display of unnatural behaviours. To further support the high-quality aspects of this enclosure the sample population had no access to enrichment devices or routines, consequently the need to substitute "add-in" enrichment items is not always required in biologically-relevant, species-specific enclosures that provide sufficient enrichment in their own right.

Robinchaud's (2011) review of several data on sitatunga activity states that the majority of a daily time budget is spent within a wetland habitat, and only a fraction of this is spent on drier areas of grassland. The same author explains that open grassland is the sitatunga's least preferred habitat, but that animals will also avoid excessively boggy areas, presumably because these are open and devoid of cover. Interestingly, to support this, the adult male studied here spent less than 0.3% of the observation period in the open marshy zone, and the adult females showed a slight preference (out of all fully aquatic areas) for zone 6, the shallow pool. Plate 3 illustrates the social and investigative behaviour that can take place when sitatunga are provided with an expanse of shallow wetland areas with a zoo enclosure.

Plate 3. Example of shallow wetlands recreated in captivity that provides an opportunity for increased behavioural diversity (author's photograph, 2011).

The structure and type of wetland habitat created for sitatunga will influence time spent within it, as well as how animals divide their time between other areas of their exhibit and the range of behaviour they perform. As highlighted by Figure 2, captive sitatunga changed their rates of feeding, resting and moving throughout the day but in an apparently different manner to that observed in the wild (Figure 3). There may be a simple explanation for the differences between resting and standing behaviour as illustrated by Figure 3. Wild sitatunga have been observed to stand motionless in reeds during midday (Owen 1970; Kingdon 1982; Robinchaud 2011); if such "habitat" is not readily available in captivity, the animals may be expressing the need to be motionless but in a different form. Therefore the function of the behaviour carries the same important need for the animal. However, removal of any threat of predation could also result in more time spent relaxed across a range of the day that would not be seen in wild animals. Similarly, climatic differences may also account for altered activity patterns in zoo housed animals in temperate parts of the world. Differences in when feeding occurs (again illustrated in Figure 3) could be used as evidence for a change in how and when captive sitatunga are fed; provision of forage in the early morning and late in the afternoon may allow for a more natural activity pattern to emerge. However, the logistics of keeper routines and the climate that zoo animals are kept in may not always allow this to be practicable.

Several areas of the exhibit are poorly utilised by the sitatunga, and one zone is not used at all (see Table 3); consequently, work could be undertaken to improve the nature of these zones to the exhibit as a whole as well as further investigate the reasons for their avoidance by the antelope. The three waterfalls in the exhibit are not well-used and such a feature is not evident in the sitatunga's natural habitat. Re-modelling of defunct areas and more space given over to favoured areas (e.g. the shallow pools and reed beds) would help increase the use of more areas of the exhibit.

Social pressures on individuals within the group could also affect type and duration of behaviour performed, as well as the area of the enclosure the animal is able to frequent. The distinct difference (Figures 2 and 5) between the time spent in biologically-relevant zones by the young male and the rest of the group could be suggested of an individual being forcibly pushed out of the herd and into the remaining, unoccupied areas of the enclosure. Popp (1982) bemoans the lack of information available on sitatunga, in both a wild and captive state, and expresses his wish that captive research will lead into better husbandry and informed management of this species. Consequently, the importance of group dynamics, hierarchy and sociality is hard to infer. Thirty years later, published information is still scarce and as such it is hoped that this paper adds to a gap in our knowledge of more advanced *Tragelaphus* antelope husbandry.

Conclusions

1. The non-random distribution of sitatunga around the enclosure demonstrates a preference for specific areas that the animals consider important to them.
2. Sitatunga show a daily rhythmic change to activity that in parts is reminiscent of wild animals but that can also show deviation away from what is considered a natural time budget.
3. The welfare of the sitatunga appears good with no display of stereotypic, unwanted behaviours.
4. Feeding/foraging, resting/rumination and standing appear the most biologically-important behaviour shown by captive sitatunga, characteristics that mirror the behaviours recorded in wild animals. Behaviours that occupy the majority of the animal's time should be enabled in its enclosure both at the "normal" time of day and in a habitat that allows the behaviour to be performed in its entirety.
5. Provision of more tall grass and reedy areas in sitatunga exhibits would be beneficial to behavioural performance, sitatunga welfare and the development of a more interesting and relevant public exhibit.
6. Reduction in the area of short grass provided, or the use of short grass pathways through areas of taller vegetation could help replicate the type of environment selected by wild sitatunga.

Acknowledgements

The authors wish to thank all of the staff at Cricket St Thomas Wildlife Park for allowing access to the sitatunga, Ms L. Clifton-Bumpas and Mrs S. Reynolds for proof-reading the manuscript, and Mr R. Coffin and Mr D. Wright for assistance with statistical analyses.

References

Antelope TAG. (2006). *AZA Minimum husbandry guidelines for keeping antelopes and gazelles in captivity*.

Bashaw M.J., Tarou L.R., Maki T.S., Maple T.L. (2001). A survey assessment of variables related to stereotypy in captive giraffe and okapi. *Applied Animal Behaviour Science* 73 (3): 235-247.

Baxter E., Plowman A.B. (2001). The effect of increasing dietary fibre on feeding, rumination and oral stereotypies in captive giraffe (*Giraffa camelopardalis). Animal Welfare* 10: 281-290.

Blowers T.E., Waterman J.M., Kuhar C.W., Bettinger T.L. (2012). Female Nile hippopotamus (*Hippopotamus amphibius*) space use in a naturalistic exhibit. *Zoo Biology* 31: 129-136.

Clark F.E., Fitzpatrick M., Hartley A., King A.J., Lee T., Routh A., Walker S., George K. (2012). Relationship between behaviour, adrenal activity, and environment in zoo-housed western lowland gorillas (*Gorilla gorilla gorilla*). *Zoo Biology* 31: 306-321.

Day J.E.L., Kyriazakisa I., Lawrence A.B. (1995). The effect of food deprivation on the expression of foraging and exploratory behaviour in the growing pig. *Applied Animal Behaviour Science* 42: 193-206.

de Jonge F.H., Tilly S-L., Baars A.M., Spruijt B.M. (2008). On the rewarding nature of appetitive feeding behaviour in pigs (*Sus scrofa*): Do domesticated pigs contrafreeload? *Applied Animal Behaviour Science* 114: 359-372.

Delany M.J., Happold D.C.D. (1979). *Ecology of African mammals*. London, UK: Longman Group Ltd.

Dickens M. (1955). A statistical formula to quantify the "spread of participation" in group discussion. *Speech Monographs* 22: 28–31.

Duncan I.J.H. (1998). Behaviour and behavioural needs. *Poultry Science* 77: 1766–1772.

Estes R.D. (1991). *The behaviour guide to African mammals*. Berkeley, USA: University of California Press.

Estevez I., Christman M.C. (2007). Analysis of the movement and use of space of animals in confinement: The effect of sampling effort. *Applied Animal Behaviour Science* 97: 221-240.

Fabregas M.C., Guillen-Salazar F., Garces-Narro, C. (2012). Do naturalistic enclosures provide suitable environments for zoo animals? *Zoo Biology* 31: 362-373.

Games, I. (1983). Observations on the sitatunga *Tragelaphus spekii selousi* in the Okavango delta of Botswana. *Biological Conservation* 27: 157-170.

Hebert P.L., Bard K. (2000). Orang utan use of vertical space in an innovative habitat. *Zoo Biology* 19:239-251

Hedeen S.E. (1983). The use of space by lowland gorillas (*Gorilla g. gorilla*) in an outdoor enclosure. *Ohio Journal of Science* 83(4): 183-185.

Kingdon J. (1982). *East African mammals; an atlas of evolution in Africa (volume III part C. bovids)*. London, UK: Academic Press.

IUCN SSC Antelope Specialist Group. (2008). *Tragelaphus spekii*. In: IUCN 2011. *IUCN Red List of Threatened Species*. Version 2011.2. www.iucnredlist.org.

MacDonald D. (2001). *The new encyclopedia of mammals* (2nd edition). Oxford, UK: Oxford University Press.

Mallapur A., Qureshi Q., Chellam R. (2002). Enclosure design and space utilization by Indian leopards (*Panthera pardus*) in four zoos in southern India. *Journal of Applied Animal Welfare Science* 5(2): 111-124.

Mason G.J., Mendl M. (1997). Do the stereotypies of pigs, chickens and mink reflect adaptive species differences in the control of foraging? *Applied Animal Behaviour Science* 53: 45-58.

Melfi V.A. (2009). There are big gaps in our knowledge, and thus approach, to zoo animal welfare: a case for evidence-based zoo animal management. *Zoo Biology* 28: 1-15.

Owen R.E.A. (1970). Some observations of the sitatunga in Kenya. *East African Wildlife Journal* 8: 181-196.

Plowman A.B. (2003). A note on a modification of the spread of participation index allowing for unequal zones. *Applied Animal Behaviour Science* 83(4): 331-336.

Popp J.W. (1982). Observations on the behaviour of captive Sitatunga (*Tragelaphus spekii*). *Zoo Biology* 1(1): 59-63.

Reinhardt V., Liss C., Stevens C. (1996). Space requirement stipulations for caged non-human primates in the United States: a critical review. *Animal Welfare* 5: 361-372.

Robinchaud W.G. (2011). Family Bovidae (sitatunga). In: Wilson, D.E. and Mittermeier R.A (eds). *Handbook of the Mammals of the World* (volume 2). Barcelona, Spain: Lynx Edicions.

Rose P.E., Roffe S.M. (2013). A case study of Malayan tapir (*Tapirus indicus*) husbandry practice across 10 zoological collections. *Zoo Biology* (article in press), DOI: 10.1002/zoo.21018.

Ross S.R., Lukas K.E. (2006). Use of space in a non-naturalistic environment by chimpanzees (*Pan troglodytes*) and lowland gorillas (*Gorilla gorilla gorilla*). *Applied Animal Behaviour Science* 96: 143-152.

Ross S.R., Schapiro S.J., Hau J., Lukas K.E. (2009). Space use as an indicator of enclosure appropriateness: A novel measure of captive animal welfare. *Applied Animal Behaviour Science*, 121 (1): 42-50.

Ross S.R., Calcutt S., Schapiro S.J., Hau, J. (2011). Space use selectivity by chimpanzees and gorillas in an indoor-outdoor enclosure. *American Journal of Primatology* 73: 197-208.

Seidensticker J., Forthman D.L. (1998). Evolution, ecology and enrichment: basic considerations for wild animals in zoos. In: Shepherdson, D.J., Mellen, J.D. and Hutchins, M. (eds). *Second nature, environmental enrichment for captive animals*. Washington, DC: Smithsonian Institution Press.

Skinner J.D., Chimimba C.T. (2006). *The mammals of the South African Subregion* (3rd edition). Cambridge, UK: Cambridge University Press.

Starin E.D. (2000). Notes on sitatunga in the Gambia. *African Journal of Ecology* 38(4): 339-342.

WAZA (n.d.). Sitatunga in the zoo. Available from: *http://www.waza.org/en/zoo/choose-a-species/mammals/eventoed-ungulates-artiodactyla/antelopes/tragelaphus-spekii*

Wronski T., Apio A., Plath, M. (2006). Activity Patterns of bushbuck (*Tragelaphus scriptus*) in Queen Elizabeth National Park. *Behavioural Processes* 73: 333-341.

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Research article

Assessment of the microbial communities associated with white syndrome and brown jelly syndrome in aquarium corals

Michael Sweet[1], Jamie Craggs[2], James Robson[2] and John Bythell[1]

[1]*School of Biology, Ridley Building, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK*
[2]*Horniman Museum & Gardens, London Road, Forest Hill, London, SE23 3PQ, UK*
**Corresponding author: michael.sweet@newcastle.ac.uk*

Keywords:
Microbial communities, white syndrome, brown jelly syndrome, corals

Article history:
Received: 02 November 2012
Accepted: 12 May 2013
Published online: 01 July 2013

Abstract

Bacterial and ciliate assemblages associated with aquarium corals displaying white syndrome (WS) and brown jelly syndrome (BJS) were investigated. Healthy (n = 10) and diseased corals (WS n = 18; BJS n = 3) were analysed for 16S rRNA gene bacterial diversity, total bacterial abundance and vibrio-specific 16S rRNA gene abundance. This was conducted alongside analysis of 18S rRNA gene sequencing targeting ciliates, a group of organisms largely overlooked for their potential as causal agents of coral disease. Despite significant differences between healthy and diseased corals in their 16S rRNA gene bacterial diversity, total bacterial abundance and vibrio-specific rRNA gene abundance, no dominant bacterial ribotypes were found consistently within the diseased samples. In contrast, one ciliate morphotype, named Morph 3 in this study (GenBank Accession Numbers JF831358 for the ciliate isolated from WS and JF831359 for the ciliate isolated from BJS) was observed to burrow into and underneath the coral tissues at the disease lesion in both disease types and contained algal endosymbionts indicative of coral tissue ingestion. This ciliate was observed in larger numbers in BJS compared to WS, giving rise to the characteristic jelly like substance in BJS. Morph 3 varied by only 1 bp over 549 bp from the recently described Morph 1 ciliate (GenBank Accession No. JN626268), which has been shown to be present in field samples of WS and Brown Band Disease (BrB) in the Indo-Pacific. This result indicates a close relationship between these aquarium diseases and those observed in the wild.

Introduction

The aquarium industry is a significant global enterprise worth $200-300 million annually (Wabnitz *et al.* 2003), with an estimated 11-12 million pieces of coral traded annually (Vincent 2006). High mortality rates of corals in aquaria are therefore a major concern (Sweet *et al.* 2011a). While many cases of coral disease in aquaria are non specific and likely due to deteriorating water quality rather than primary pathogen infections (Borneman and Lowrie 2001; Sweet *et al.* 2011a), there are a number of specific diseases with clearly defined signs (reviewed by Sweet *et al.* 2011a). These include brown jelly syndrome (BJS) and Red Slime Algae which have no reported parallels in nature, and white syndrome (WS), which has similar macroscopic signs to the disease of the same name in the wild (Fig 1). As in the wild, WS within aquariums may progress at different rates (reported to be 0.1 cm per day to 10 cm per hr). The coral diseases with similar macroscopic signs but faster rates of lesion progression are variously known as Rapid Tissue Necrosis, Shut Down Reaction, and Stress Related Necrosis (Hormansdorfer *et al.* 2000; Borneman and Lowrie 2001; Luna *et al.* 2007; Luna *et al.* 2010; Sweet *et al.* 2011a). The slower rates of progression observed in WS within aquaria are similar to those reported for WS in the field (Ainsworth *et al.* 2007; Andersen *et al.* 2010; Luna *et al.* 2010; Work and Aeby 2011).

WS is defined as progressive, full-thickness tissue loss from the coral with a sharp demarcation between the apparently healthy tissue and the denuded white skeleton. Willis *et al.* (2004) defined the term WS to encompass any disease with unknown etiology which showed these particular pathological field signs. Although several studies have implicated specific bacterial pathogens to be the cause of WS (Sussman *et al.* 2008; Luna *et al.* 2010), there has been no evidence of a significant population of bacteria at the disease lesion interface or classical signs of bacterial-induced necrosis (Ainsworth *et al.* 2007; Work and Aeby 2011). Recently, a group of organisms known as ciliates have been shown to be present at the disease lesion interface of WS. These ciliates were shown to ingest the coral tissue and it was suggested that they are responsible for the pathology of these diseases (Sweet and Bythell 2012). This ciliate community was also shown to be similar to that of Brown Band Disease (BrB), another common disease found to occur on the Great Barrier Reef (Sweet and Bythell 2012).

BJS in aquarium corals has been strongly linked to a ciliate, commonly referred to as *Helicostoma nonatum* (Hummon 2008), which has only recently been assigned to the same genus as that of two ciliates in WS and BrB, *Philaster* (Sweet *et al.* 2011a). This study therefore describes both the ciliate and bacterial communities of WS and BJS in aquarium corals using culture-independent (18S and 16S rRNA gene) techniques. Diseased samples were taken opportunistically as they arose in the aquariums, and compared to non-diseased samples collected at the same time from the same aquarium.

Methods

Sample collection

Corals displaying signs of WS were collected from three locations; Horniman Museum and Gardens aquarium in London, the Zoological Society of London (ZSL) aquarium and our own aquarium at Newcastle University. These diseased samples were compared to apparently healthy corals from the same aquarium systems. Healthy samples (n = 10) and diseased samples (n = 18) of six different species (Table 1) were collected from the various aquariums (Fig 1, Table 1). A further three samples of coral exhibiting symptoms of brown jelly syndrome (Fig 1f), were acquired from ZSL and The Deep (Hull, UK) for analysis of the ciliates previously proposed as the causal agent of this disease. Coral samples were photographed before removal from the aquarium then placed in 50 ml falcon tubes with 100% EtOH and stored at -20°C until extraction and further analysis. Samples were centrifuged at 20,000 rpm for 20 min to concentrate the tissue slurry, 1000 µl of which was subsequently used for DNA extraction using QIAGEN DNeasy Blood and Tissue kits (Sweet *et al.* 2011b) with an added step to concentrate the lysate using a vacuum centrifuge for 2 h at 24° C.

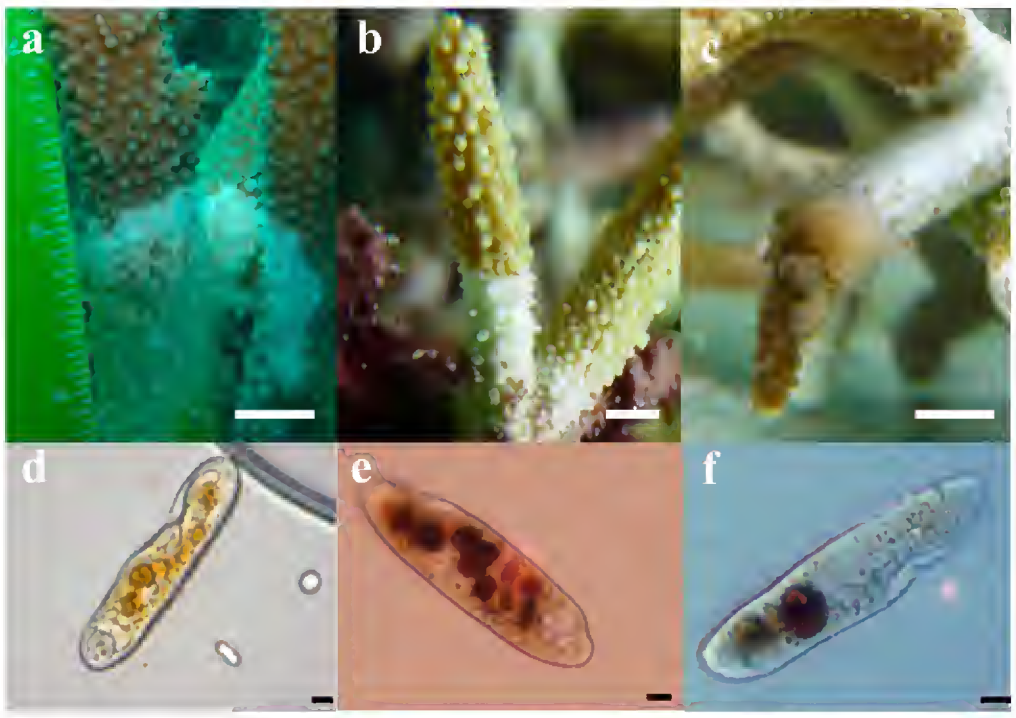

Figure 1. Sub set of samples exhibiting white syndrome (WS) and the dominant ciliates associated with the lesion interface; (a) wild *Acropora muricata*, from Heron Island on the Great Barrier Reef, (b) *Acropora* sp. from aquarium at Zoological Society of London (ZSL), (c) *Acropora* sp. showing brown jelly syndrome (BJS), (d) *Philaster* sp. found in corals exhibiting white syndrome at Heron Island (e) *Philaster* sp. found in corals exhibiting white syndrome in aquarium; (f) *Philaster* sp. found in corals exhibiting brown jelly syndrome in aquarium [note: these protozoa were previously miss-identified as *Helicostoma notatum*]. Sequence data provided analysis to genus level with phylogenetic analysis showing differences and similarities between these sequences (Figure 6). Morphologically these ciliates can be identified by a slender body, 60–200 x 20–60 µm in vivo, variable in outline from cylindrical to fusiform; anteriorly narrowed and conspicuously pointed. Length of the buccal field is ~ 40–50% of the body, cytostome is conspicuous and deeply sunk. Macronucleus band-like, twisted and positioned centrally along cell median with several micronuclei attached to it. One small, terminally located contractile vacuole. Approximately 50 somatic kineties composed of monokinetids, with cilia c. 7–10 m long; oral cilia c. 10–15 mm long; caudal cilium 12–15 mm in length. Paroral membrane L-shaped, on right of oral cavity, slightly oblique to main body axis. Locomotion by fast, spiral swimming while rotating irregularly about its main body axis, motionless for short periods when feeding. White Scale bars (a-c) = 1cm. Black Scale bars (d-f) = 10µm.

Bacterial diversity, DNA extraction, amplification and DGGE analysis

Bacterial partial 16S rRNA gene fragments were amplified using standard prokaryotic primers (357F) (5′-CCTACGGGAGGCAGCAG-3′) and (518R) (5′-ATTACCGCGGCTGCTGG-3′). The GC-rich sequence 5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA GCA CGG GGG G-3′ was incorporated in the forward primer 357 at its 5′ end to prevent complete disassociation of the DNA fragments during DGGE. Thirty PCR cycles were performed at 94°C for 30 seconds, 53°C for 30 seconds and 72°C for 1 min and a final extension at 72°C for 10 min (Sanchez *et al.* 2007). Three independent 10 µl PCR reactions were used, each containing 1.5 mM $MgCl_2$, 0.2 mM dNTP (PROMEGA), bovine serum albumin (BSA, 400 ng µl^{-1}), 0.5 mM of both the forward and reverse primers, 2.5 U of Taq DNA polymerase (QBiogene), incubation buffer, and 20 ng of template DNA (Siboni *et al.* 2007). These replicate PCR's for each sample were then combined and cleaned using QIAGEN QIAquick PCR purification kits, reducing the final volume to 15 µl in Sigma molecular grade H_2O. All reactions were performed using a Hybraid PCR Express thermal cycler. PCR products were verified by agarose gel electrophoresis (1.6% (w/v) agarose) with ethidium bromide staining and visualized using a UV transilluminator.

Sample Number	Species	Location	Health status	Rate of tissue loss (mm$^{2/}$day)
1	*Acropora pulchra*	HM	Healthy	NA
2	*Acropora formosa*	HM	Healthy	NA
3	*Acropora sp*	HM	Healthy	NA
4	*Acropora sp*	ZSL	Healthy	NA
5	*Pocilliopora damicornis*	NU	Healthy	NA
6	*Pocilliopora damicornis*	NU	Healthy	NA
7	*Acropora formosa*	NU	Healthy	NA
8	*Acropora formosa*	NU	Healthy	NA
9	*Pocilliopora damicornis*	HM	Healthy	NA
10	*Pocilliopora damicornis*	HM	Healthy	NA
11	*Acropora sp*	HM	WS^{1}	0.2
12	*Acropora sp*	HM	WS^{1}	0.2
13	*Seriatopora hystrix*	HM	WS^{2}	0.2
14	*Montipora capricornis*	HM	WS^{3}	0.5
15	*Pocilliopora damicornis*	HM	WS^{1}	1.6
16	*Acropora sp*	ZSL	WS^{1}	10
17	*Pocilliopora damicornis*	ZSL	WS^{1}	10
18	*Euphyllia yaeyamnmesis*	ZSL	WS^{1}	10
19	*Pocilliopora damicornis*	NU	WS^{1}	10
20	*Pocilliopora damicornis*	NU	WS^{1}	1.9
21	*Acropora sp*	ZSL	WS^{1}	10
22	*Acropora sp*	ZSL	WS^{1}	10
23	*Acropora sp*	ZSL	WS^{1}	10
24	*Acropora sp*	ZSL	WS^{1}	10
25	*Acropora sp*	ZSL	WS^{1}	11
26	*Acropora sp*	NU	WS^{1}	16
27	*Acropora formosa*	NU	WS^{1}	10
28	*Hydnophora rigida*	HM	WS^{1}	28
29	*Acropora sp*	ZSL	BJS	20
30	*Acropora sp*	Deep	BJS	15
31	*Acropora sp*	Deep	BJS	20

^{1}WS starting at the extremities; ^{2}WS starting along the branch; ^{3}WS patchy distribution

Table 1. Sample set illustrating species and location utilised in this study, health status relates to described status of coral at time of samples. Healthy represent apparently healthy specimens, WS represent white syndrome disease signs, BJS brown jelly syndrome HM = Horniman Museum; ZSL = Zoological society of London; NU = Newcastle University, Deep = The Deep in Hull. Rates of tissue loss are estimates by personal observations in tank systems prior to transportation up to Newcastle for analysis and may not be accurate. Pers. obs. at ZSL showed an average advance rate of the tissue lesion of 10 mm^{2} day^{-1} from the base, until ~10 cm from the tip then the rate would be accelerated and the coral would appear to 'shut down' and die off overnight ~100mm^{2}

DGGE was performed using the D-Code universal mutation detection system (Bio-Rad). Bacterial PCR products were resolved on 10 % (w/v) polyacrylamide gels that contained a 30–60 % denaturant gradient for 13 h at 60 °C and a constant voltage of 50 V. Gels were stained with a concentrated solution of 9 µl Sybr® Gold (Sigma) in 50 µl of 1X TAE poured directly onto the gel surface, covered and left in the dark for 20 min then further washed in 500 ml 1X TAE for 30 min and visualized using a UV transilluminator. Bacterial operational taxonomic units (OTUs), were defined from DGGE band-matching analysis using BioNumerics 3.5 (Applied Maths BVBA) following methods described by Guppy and Bythell (2006). Standard internal marker lanes were used to allow for gel-to-gel comparisons. Tolerance and optimisation for band-matching was set at 1%. OTUs of interest (those which explained the greatest differences/similarities between samples), were identified by sequence analysis. Bands were excised from DGGE gels, left overnight in Sigma molecular grade water, vacuum centrifuged, re-amplified with the appropriate primer set, labelled using Big Dye (Applied Biosystems) transformation sequence kits and sent to Genevision (Newcastle University, UK) for sequencing.

Ciliate diversity, DNA extraction, amplification and DGGE analysis

Single cell isolates

Corals were viewed under a binocular microscope and single cell isolates of ciliate morphotypes were sampled using a 10 µl pipette and stored in 1.5 ml micro-centrifuge tubes containing 100 µl EtOH at -20°C. DNA was extracted from the ethanol-fixed single isolates following a modified Chelex Extraction (Walsh *et al.* 1991). The samples were centrifuged for 2 min at 20,000 g and washed 3 times in sigma water with a centrifuge step between each wash. After the last wash 50 µl of a 5 % Chelex 100 (sigma) solution and 15 µl of proteinase K (20 mg/ml) were added to the cell isolate. Samples were incubated overnight in a water bath at 56°C, vortexed for 10 sec, boiled in a 100°C water bath for 10 min, vortexed again for 10-20 sec, and finally centrifuged for 3 min at 16,000 g; 40 µl of the supernatant was taken off and put in a fresh micro-centrifuge tube for use in subsequent PCR reactions.

PCR amplifications of single cell isolates were carried out using 20 µl reaction mixtures (final PCR buffer contained: 1 mM $MgCl_2$, and 1 U Taq DNA polymerase (QBiogene); 100 µM deoxynucleoside triphosphates; 0.2 µM of each of the forward and reverse primers; and 0.4 % bovine serum albumin, with 20 ng of template DNA. All reactions were performed using a Hybaid PCR Express thermal cycler. PCR products were analysed by agarose gel electrophoresis [1.6 % (w/v) agarose] with ethidium bromide staining and visualized using a UV transilluminator. A nested PCR was used to yield best results, initially with the universal 18S eukaryotic primer pairs f4617 (5'-TCCTGCCAGTAGTCATATGC-3') (T. Tengs *pers. commun.*) and r4618 (5'-TGATCCTTCTGCAGGTTCACCTAC-3'). PCR protocol was; 95°C for 3 min, then 40 cycles of (95°C for 30 sec, 55°C for 30s and 72°C for 90s); followed by 5 min at 72°C (Oldach *et al.* 2000). Then with internal ciliate specific primers 384f-cil (5'-YTBGATGGTAGTGTATTGGA-3') and 1147r-cil (5'-GACGGTATCTRATCG TCTTT-3'). PCR protocol was; 94°C for 5 min, then 30 cycles of (94°C for 45 sec, 55°C for 60 sec and 72°C for 90 sec); followed by 72°C for 7 min (Dopheide *et al.* 2008). Sequencing was carried out as above using Big Dye sequence kits and cleaned with an EtOH precipitation method and sequenced at Genius limited, Newcastle University.

Ciliate DGGE

The same coral samples (as used for the bacterial analysis) were analysed for their ciliate diversity. Partial ciliate 18S rRNA gene ribotypes were amplified with a single-round PCR approach (Jousset *et al.* 2010). Three replicate PCR reactions were carried out as above (final PCR buffer contained: 1 mM $MgCl_2$, and 1 U Taq DNA polymerase (QBiogene); 100 µM dNTPs; 0.2 µM of each of the forward and reverse primers; and 0.4% BSA, with 20 ng of template DNA. Primers Cil-f (5'-TGGTAGTGTATTGGACWACCA-3') with a 36-bp GC clamp (Muyzer and Smalla 1998) attached to the 5' end and CilDGGE-r (5'-TGAAAACATCCTTGGCAAATG-3') were used. Initial denaturation was at 94°C for 5 min, followed by 26 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min and a final elongation of 10 min at 72°C to reduce double bands in the DGGE patterns (Janse *et al.* 2004). PCR products of the 3 replicate samples were combined and subjected to DGGE on a D-code system (Bio-Rad) with 0.75 mm thick 6 % polyacrylamide gels in 1 X TAE buffer. Electrophoresis was carried out for 16 h at 60°C and 50 V in a linear 32 to 42 % deionised formamide denaturing gradient. Gels were stained as above and OTUs of interest were similarly identified by band excision and sequencing.

Quantitative PCR (qPCR) targeting Vibrio sp.

Quantitative PCR (qPCR) was conducted on an Engine Opticon® 2 system in order to test whether *Vibrio* sp. relative 16S rRNA abundance differed between a random subset of the diseased samples (n = 10) and healthy coral samples (n = 10). qPCR assays were standardised using a serial dilution ($10^9 - 10^1$ CFU ml^{-1}; 3 replicates per dilution) of pure cultures of *Vibrio harveyi* (NR043165) and run on the qPCR machine to determine threshold cycles. *Vibrio*-specific primers 567F (5'-GGCGTAAAGCGCATGCAGGT-3') and 680R (5'-GAAATTCTACCCCCCTCTACAG-3') (Thompson *et al.* 2004; Sweet *et al.* 2010), were used for all samples. qPCR reaction mixtures totalled 25 µl and consisted of 12.5 µl of 2X Quantitect® Sybr® Green 1 supermix (Qiagen), 1.25 µl each of 0.5 mM forward and reverse primers, 50 ng DNA and 9.5 µl Sigma molecular grade water. Each set of samples included a negative control, in which water was substituted for the DNA sample. qPCR was performed with an initial activation step of 15 min at 95°C, followed by 39 cycles (94°C for 15 sec, 58°C for 30 sec, primer annealing at 58°C for 30 sec). The fluorescent product was detected after each extension. Following amplification, melting temperature analysis of PCR products was performed to determine the specificity of the PCR. The melting curves were obtained by slow heating at 0.5°C sec^{-1} increments from 50 to 90°C, with continuous fluorescence recording.

Total bacterial abundance

To estimate bacterial abundance, 1000 µl of tissue slurry was filtered through a 0.22 µm black polycarbonate filter and fixed with 100 µl of paraformaldehyde until analysis (Fuhrman *et al.* 2008). These filters were stained with 100 µl DAPI solution (final concentration 5 µg ml^{-1}) for 10 min, rinsed with Phosphate Buffer Solution (Yu *et al.* 1995; Weinbauer *et al.* 1998; Yamaguchi *et al.* 2007), and viewed under epifluorescence microscopy using a DAPI-specific filter set. Counts on 50 fields of view (FOV), were taken using an automatic cell counter (Cell C; (Selinummi *et al.* 2005). The parameters were set to exclude any objects smaller than 0.03 $µm^2$ and anything larger than 0.7 $µm^2$. Counts were scaled up to the total area of the filter and calculated to give total bacterial abundance per volume of tissue on the diseased corals (cells cm^3). Total amount of diseased tissue rather than complete coral nubbin surface area was used to account for the varying amount of tissue on the diseased samples as this could not be standardised at time of collection. Counts of n = 3 tissue subsamples were taken from each coral and averaged to provide a cell density per sample.

Statistical analysis

Analysis of Similarity (ANOSIM) tests based on Bray-Curtis similarities (Clarke and Warwick 2001) were used to test for differences in the bacterial 16S and ciliate 18S rRNA gene DGGE profiles associated with diseased and healthy corals. A non-metric multidimensional scaling (MDS) analysis was used to represent each sample type on a 2-D plot (Clarke and Warwick 2001). An analysis of contribution to similarities (SIMPER) based on DGGE analysis with both relative band intensity and presence absence was performed to determine which 16S and 18S rRNA gene OTUs best explained dissimilarities among sample types that were statistically different. The abundance of bacteria (total bacterial counts) was compared between healthy and diseased samples with a one-way ANOVA. Data met the assumptions of normality and equality of variances (Anderson Darling and Levene's tests respectively). For qPCR a standard curve delineating threshold (CT) values of *V. harveyi* against number of *V. harveyi* (CFU ml^{-1}) in pure cultures gave a standard curve ($R^2 = 0.996$) allowing quantification of the assay. Calculations were then based on 1 CFU relating to a genome copy. One way ANOVA was used to compare between healthy and diseased samples for *Vibrio* fold difference.

Results

Significant differences based on profiling of bacterial communities using DGGE analysis (PERMANOVA, R = 0.494, p = 0.001), were found between the bacterial 16S rRNA gene diversity of healthy and diseased coral samples (Fig 2, 3). Within healthy coral samples, there were no significant differences between the three aquariums where samples were sourced from, or between species of coral (two-way crossed ANOSIM, R = 0.29, p = 0.2 and R = 0.187, p = 0.28, respectively). Similarly no significant differences were detected between diseased samples with different rates of progression and disease type (WS or BJS) (two-way crossed ANOSIM, R = 0.19, p = 0.09 and R = 0.22, p = 0.12 respectively, Fig 3a,b).

There was a significant difference in bacterial abundance associated with healthy versus diseased corals (ANOVA, df = 1, F = 5.15, p = 0.03), with mean bacterial abundance increasing in diseased corals (6.1 ± 0.52 (SD) x 10^7 cells cm^{-3}), compared to that of healthy samples (3.5 ± 0.23 (SD) x 10^7 cells cm^{-3}) (Fig 4a). *Vibrio* abundance also

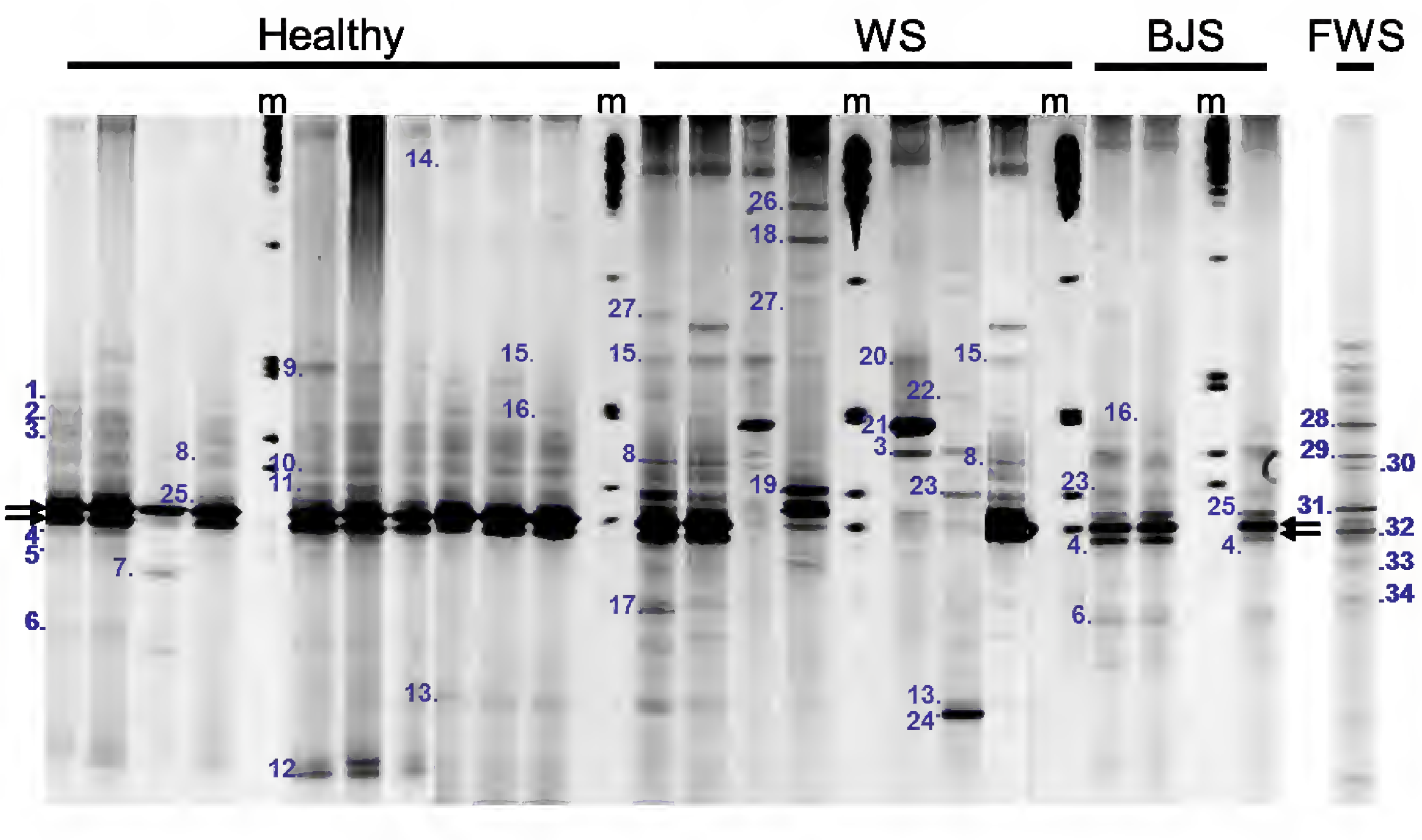

Figure 2. DGGE analysis of bacterial 16S rRNA gene diversity from healthy and diseased corals. Healthy = corals showing no signs of distress or symptoms of disease at time of collection; WS = white syndrome collected from aquariums, corals showing signs of some form of tissue necrosis similar to that known as rapid tissue necrosis or white syndrome. FWS = field white syndrome from Heron Island as reported in Sweet and Bythell 2012, used as a reference to show similarities and differences between those in aquarium and those in the wild. Arrows indicate corals symbiotic algae, *Symbiodinium* sp. Band numbers (1 – 27 in blue) relates to ribotypes excised from the cell and sequenced (Table 2). Intermittent marker lanes (m) allowed gel to gel comparison using the software BioNumerics.

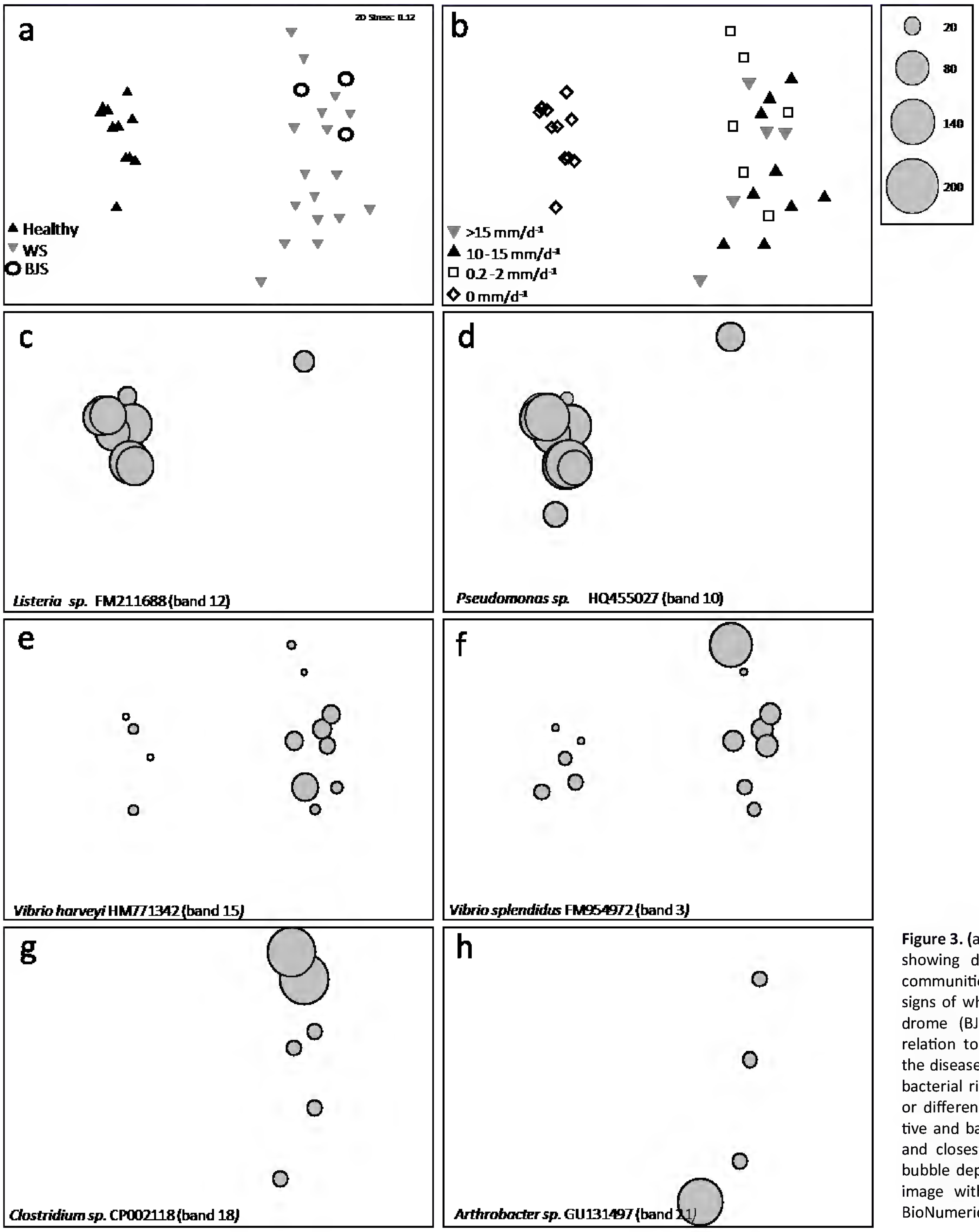

Figure 3. (a) Multidimensional scaling (MDS) plot showing differences in 16S rRNA gene bacterial communities from healthy corals to corals showing signs of white syndrome (WS) and brown jelly syndrome (BJS). (b) shows grouping of samples in relation to variation in their lesion progression of the disease (mm per day) (c-h) show 16S rRNA gene bacterial ribotypes that caused greatest similarities or differences between sample types (closest relative and band no in relation to DGGE image [Fig 2] and closest match on GenBank [Table 2]. Size of bubble depicts intensity of band/ribotype on DGGE image within individual samples calculated using BioNumerics.

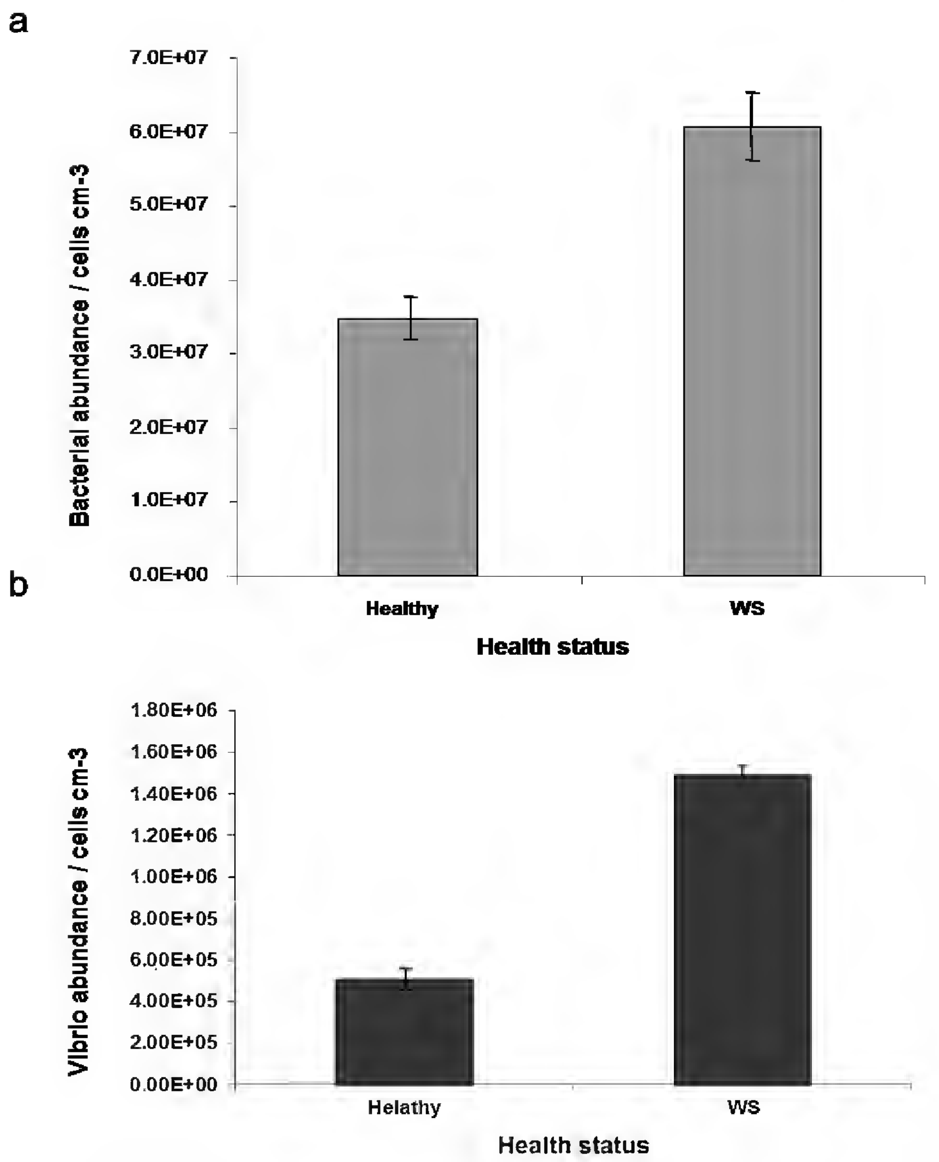

Figure 4. (a) Total bacterial abundance of healthy and diseased corals within aquarium, acquired from filtered tissue slurry and DAPI staining, counts made using the automatic cell counter Cell C; (b) total relative *Vibrio* sp. per cm^3 of tissue present within healthy and diseased coral samples, acquired from quantitative PCR (see materials and methods) for ten replicate qPCR runs for both healthy and diseased samples. Error bars represent SE for collective mean.

increased significantly (ANOVA, df = 1, F = 4.46, p = 0.043) in diseased coral compared to healthy (1.5 ± 0.48 (SD) x 10^6 cell cm^{-3} and 5.3 ± 0.37 (SD) x 10^5 cells cm^{-3} respectively) (Fig 4b).

There were several bacterial ribotypes (a *Pseudomonas* sp. GenBank closest relative HQ455027 and a *Listeria* sp. GenBank closest relative FM211688) which reduced in dominance in diseased tissues compared to healthy samples (Fig 2 and 3c,d). Whilst two other ribotypes were completely absent from diseased samples, including a *Pelobacter* sp. (GenBank closest relative CP000482) and a *Acidiobacterium* sp. (GenBank closest relative EF076073) (Table 2). Several bacterial ribotypes increased in relative 16S rRNA gene abundance in some diseased samples including ribotypes similar to a *Pseudovibrio* sp. (GenBank closest relative GU826592), a *Cyanobacterium* sp. (GenBank closest relative HQ230344), a *Arcobacter* sp. (GenBank closest relative HM584709) and two *Vibrio* sp. (GenBank closest relative HM771342 and FM954972; Fig 3e and f respectively). Three bacterial ribotypes were absent from healthy specimens and dominant in some diseased samples, including a *Clostridium* sp. (GenBank closest relative CP002118; Fig 3g), a *Arthrobacter* sp. (GenBank closest relative GU131497; Fig 3h) and a *Microbacterium* sp. (GenBank closest relative EU249984) (Fig 2 and 3, Table 2). However, there were no bacterial ribotypes consistently present in all cases of disease (Fig 2).

Ciliates were only observed on diseased tissues and completely absent in healthy corals. This observation was supported by 18S rRNA gene analysis which showed a diversity of ciliate ribotypes in diseased samples and produced no PCR product in healthy samples (Fig 5). At least 7 ciliate ribotypes were consistently present in all diseased corals. These included ribotypes similar to a *Pseudokeronopsis* sp. (GenBank closest relative HQ228545), a *Aspidisca* sp. (GenBank closest relative AF305625), a *Philaster* sp. (GenBank closest relative FJ648350), a *Glauconema* sp. (GQ214552), a *Paradiscocephalus* sp. (GenBank closest relative EU684746), a *Licnophora* sp.

			Abundance (band intensity)		**Average dissimilarity 69.95%**	
DGGE band ID	Species ID	Closest relative (% match)	Healthy	Diseased	Contribution %	Accumulative contribution (%)
10	*Pseudomonos sp.*	HQ455027 (100%)	4.13	0.38	8.28	8.28
12	*Listerio sp.*	FM211688 (100%)	3.48	0.28	7.15	15.43
26	*Pseudovibrio sp.*	GU826592 (99%)	0.35	3.25	6.25	21.68
23	*Cyanobacterium*	HM535495 (99%)	0.52	2.21	4.07	25.75
NA	Unknown	NA	1.27	1.43	3.77	29.51
2	*Pelobacter sp.*	CP000482 (99%)	1.77	0	3.74	33.25
4	*Oscillotorioles cyanobacterium*	GU967420 (95%)	2.47	2.41	3.58	36.83
16	*cyonobacterium*	EF577468 (96%)	1.3	1.29	3.32	40.15
25	*Arcobocter sp.*	HM584709 (96%)	0.78	1.41	3.31	43.47
24	*Clostridium sp.*	AY712079 (97%)	1.99	3.43	3.29	46.76
15	*Vibrio horveyi*	HM771342 (96%)	0.67	1.43	3.28	50.04
3	*Vibrio splendidus*	FM954972 (100%)	0.81	1.31	3.23	53.27
18	*Clostridium sp.*	CP002118 (96%)	0	1.49	3.07	56.34
11	*Compylobacter sp.*	HM462470 (97%)	2.2	1.54	2.39	58.73
NA	Unknown	NA	0.58	0.94	2.11	60.84
1	*Stenotrophomonas sp.*	HM153430 (97%)	0.9	0.78	2.07	62.91
9	*Acidobacterium sp.*	EF076073 (100%)	0.98	0	2.05	64.96
6	*Chloroflexi sp.*	DQ330160 (98%)	0.72	0.39	1.96	66.91
NA	Unknown	NA	0.91	0.69	1.84	68.75
NA	Unknown	NA	0.05	0.9	1.83	70.58
27	*Morinobocter sp.*	HM141532 (98%)	0.92	0.77	1.82	72.4
13	*Pectobacterium sp.*	DQ123811 (99%)	0.43	0.69	1.81	74.21
NA	Unknown	NA	0.38	0.77	1.67	75.88
22	*Verrucomicrobiae sp.*	FN646737 (93%)	0.53	0.52	1.67	77.55
21	*Arthrobocter sp.*	GU131497 (96%)	0	0.78	1.64	79.18
19	*Microbocterium sp.*	EU249984 (98%)	0	0.79	1.64	80.82
8	Uncultured bacterium sp.	GU118719 (100%)	0.67	0.26	1.6	82.42
NA	Unknown	NA	0.13	0.77	1.57	83.99
7	*Gommo proteobocterium*	EF215814 (100%)	0.37	0.42	1.52	85.51
17	*Arcobocter sp.*	FR675876 (100%)	0.19	0.63	1.51	87.02
NA	Unknown	NA	0.15	0.69	1.5	88.52
NA	Unknown	NA	0.45	0.43	1.47	89.99
5	*Arcobocter sp.*	HQ317346 (96%)	0.49	0.25	1.32	91.31

Table 2. Closest match (GenBank ID) and identification of bacterial species from healthy and diseased corals in aquarium, sequenced from denaturing gradient gel electrophoresis (DGGE) bands. Out of a total of 44 operational taxonomic units (OTUs), 33 are represented in this table which account for 91.3% of variance between sample types. Relative abundance measurements are based on BioNumerics presence/absence and band intensity data

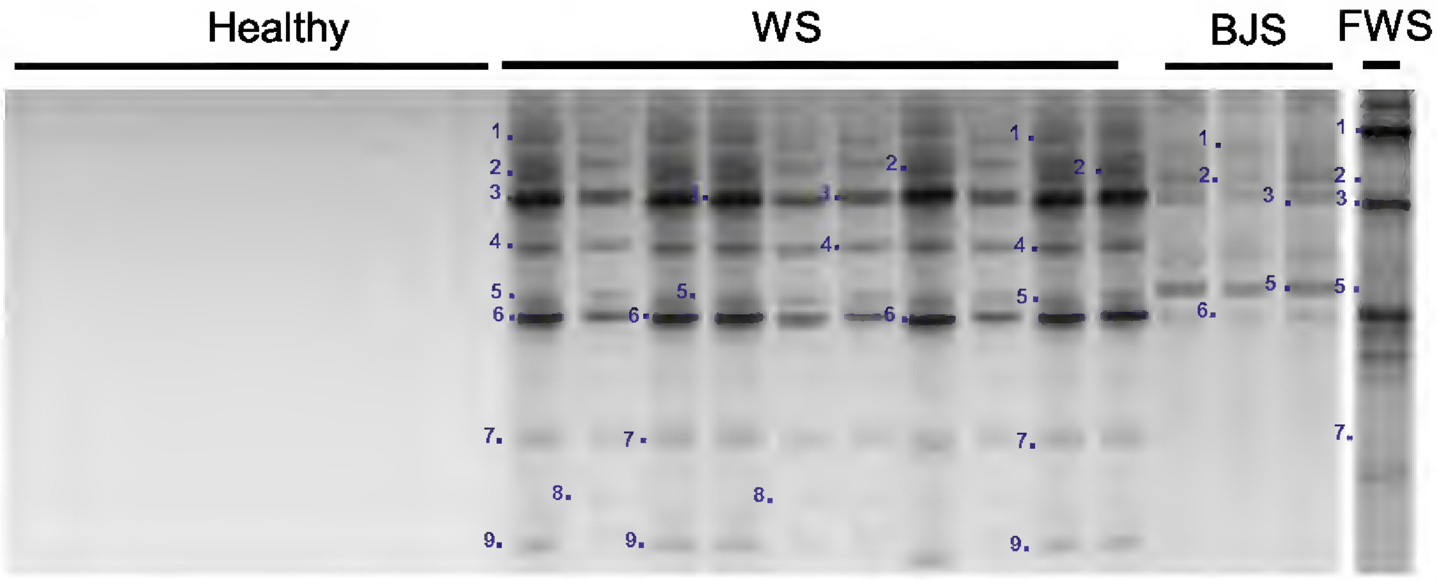

Figure 5. DGGE analysis of ciliate 18S rRNA gene diversity from healthy and diseased corals. Healthy = corals showing no signs of distress or symptoms of disease at time of collection; WS = white syndrome from aquarium, corals showing signs of some form of tissue necrosis similar to that known as rapid tissue necrosis or white syndrome. FWS = Field white syndrome from Heron Island as reported in Sweet and Bythell (2012), used as a reference to show similarities and differences between those in aquarium and those in the wild. Band numbers (1–9 in blue) relate to ribotypes excised from the cell and sequenced. 1 = *Pseudocarnopsis* sp. (HQ228545); 2 = *Aspidisca steini* (AF305625), 3 = *Philaster* sp. (FJ648350), 4 = *Hanseniaspara* sp. (JF306024), 5 = *Glauconema* sp. (GQ214552), 6 = *Parasiscocephalus elongates*, 7 = *Holostica diademento* (DQ059583), 8 = *Licnophora lyngbycola* (DQ445606) and 9 = *Chromodorina* germonica (AY854207).

(GenBank closest relative DQ445606) and a *Holosticha* sp. (GenBank closest relative DQ059583). All morphotypes detected visually and identified by sequencing single cell isolates were also detected by DGGE analysis of the ciliate community. Five of the seven ribotypes identified in WS samples matched (with >99% similarity) to those recently identified in WS in the wild (Sweet and Bythell 2012, Fig 5). These included the *Pseudokeronopsis* sp., *Aspidisca* sp., *Philaster* sp., *Glauconema* sp. and *Holosticha* sp. One DGGE band in the aquarium samples (Band 4 in Fig 5) was identified as a fungus, *Hanseniaspara* sp. (GenBank closest relative JF306024), and another (Band 9 in Fig 5) was identified as a nematode similar to *Chromodorina* sp. (GenBank closest relative AY854207), indicative of a limited amount of non-specific PCR amplification with these primers.

Only one ciliate type was observed to ingest coral tissue, as evident from the presence of coral symbiotic algae within the cell (Fig 1 d-f). This was commonly the most abundant ciliate observed and was found consistently in all WS and BJS samples. It was identified from single cell isolates as 99% similar over 549 base pairs to *Philaster digitformis* (FJ648350) and was morphologically similar to *Porpostoma notatum* (=*notate*), (Song 2000), *P. guamense* (Lobban *et al.* 2011) and *Helicostoma notatum* (the ciliate associated with BJS reviewed in Sweet *et al.* 2011a). However, single cell sequences obtained from this morphotype in aquarium disease samples were distinct (92% sequence similarity over 549 bp) from the recently submitted sequence for *Porpostoma notatum* (GenBank closest relative HM236335). Sequence data is currently lacking for the only other *Porpostoma* species reported, *Porpostoma guamense* so comparison with this species on a genetic level could not be done. Two unique GenBank accession numbers have been submitted for the morphotype in this study, JF831358 for ciliates acquired from corals with WS and JF831359 for ciliates associated with BJS. These WS and BJS sequences were closely related (>99% over 549 bp) to ribotypes recently identified in wild corals with WS (GenBank closest relative HQ204545) and Brown Band Disease (GenBank closest relative HQ204546), varying by only 2 bp over 549 (Sweet and Bythell 2012; Fig 6). Thus, the evidence strongly suggests that this same ciliate is the dominant member of WS-associated communities in both aquarium and wild samples and is also a dominant member of BJS and BrB communities (this study and Sweet and Bythell 2012).

Discussion

There was a significant difference between bacterial 16S rRNA gene diversity of healthy and diseased coral samples and a general increase in bacterial load within diseased samples, a result consistent with previous findings (Luna *et al.* 2007; Sussman *et al.* 2008; Ainsworth *et al.* 2010; Luna *et al.* 2010). In previous studies specific bacteria have been proposed as single causal agents to certain coral diseases such as WS. The most commonly referred to are from the genus *Vibrio* (Luna *et al.* 2007; Sussman *et al.* 2008; Luna *et al.* 2010). However, in this study there were no single dominant bacterial ribotypes consistently present within all diseased samples. This supports the report by Willis *et al.* (2004), in which they defined WS as a group of unidentified diseases occurring in Indo-Pacific corals with unknown etiology. Two *Vibrio* sp. and a *Pseudovibrio* sp. were found to increase in abundance within diseased specimens, making them potential candidates for pathogenesis (Sweet and Bythell 2012). However, the two *Vibrio* sp. were also detected in healthy as well as diseased samples. Several other potential pathogens were also shown to increase in relative 16S rRNA gene abundance in individual diseased samples, including ribotypes similar to; a *Cyanobacteria* sp., a *Arcobacter* sp., a *Clostridium* sp., a *Arthrobacter* sp. and a *Microbacterium* sp., however none of these specific bacteria were consistently detected in all diseased samples.

If WS was simply a case of a specific single bacterial pathogen and providing that the samples were taken at the same stage of disease progression, when casual agents would be in high abundance and high activity, you would expect to see a single dominant ribotype consistent across all samples exhibiting signs of this disease. It is therefore likely that WS is caused by an initial systemic infection by any number of potential bacterial pathogens depending on which are present when the coral becomes stressed. In addition, it should be possible to co-localise these bacterial populations with histopathology. There should be an increase in bacterial populations at the disease lesion interface and/or classic signs of bacterial induced necrosis, which up to now has not been possible (Ainsworth *et al.* 2007; Work and Aeby 2011).

In contrast, the consistent presence of the same ciliate, identified as similar to *Philaster digitformis* (FJ648350), in all diseased samples exhibiting signs of WS and those from samples exhibiting BJS, yet absent in the healthy specimens, suggests that this ciliate is an important and regularly detectable associate of the disease. Observations of the ingestion of coral tissues, and presence of coral endosymbiotic algae within the ciliate suggests that either these ciliates are directly involved in pathogenesis or alternatively they are simply associated with the necrotic tissue of the disease. Regardless, these ciliates are clearly important in the pathology of these aquarium diseases (namely the sharp band of denuded skeleton adjacent to apparently healthy tissue) a result supporting that recently found in the wild (Sweet and Bythell 2012). The ciliate identified as being involved in pathogenesis in these aquarium diseases is >99% similar to ciliates also consistently identified in both WS (HQ204545) and BrB (HQ204546) in the wild (Sweet and Bythell 2012). Several other ciliates previously identified in WS and BrB in the wild were also detected with the aquaria however were not shown to ingest coral tissues and are likely secondary colonisers. Interestingly, the dominant ciliate of BrB, first identified by Bourne *et al.* (2008), and also involved in coral tissue feeding in the wild diseases (Sweet and Bythell 2012), was absent from the aquarium diseases, suggesting that it not a necessary component of the WS pathology.

Although disease causation cannot be inferred using a purely culture independent approach, the observations of histophagy (Ainsworth *et al.* 2007; Work and Aeby 2011), together with similarities of ciliate communities in aquarium and wild corals displaying similar disease signs has led us to confirm our hypotheses reported in Sweet and Bythell (2012). In short, either; (i) opportunistic pathogenic bacterial species such as the widely-reported vibrios are the primary agents, invading healthy tissues and leading to an impaired physiological condition which allows ciliate communities to invade and proliferate at the disease lesion. Or, alternatively (ii) ciliates are the causal agents and the bacterial agents identified are either non-specific pathogens infecting the tissues that have been compromised by ciliate histophagy or are invading the dead and decomposing tissues at the lesion interface or the skeletal surfaces immediately adjacent to it. A further hypothesis can also be inferred in that the corals physiological condition is severely impaired due to either environmental stress or for example infection from other organisms not investigated in this study e.g. viruses (Davy *et al.* 2006; Marhaver *et al.* 2008) and/or fungi (Lecampionalsumard *et al.* 1995). This in turn would then allow for secondary invasion scenarios from both bacteria and ciliates.

Since potential bacterial pathogens previously linked to coral disease have routinely been detected in healthy corals in this and many other studies (Bourne and Munn 2005; Klaus *et al.* 2005; Gil-Agudelo *et al.* 2007; Arboleda and Reichardt 2009; Kvennefors *et al.* 2010; Luna *et al.* 2010), it is essential that studies assessing disease causation by techniques such as pure culture inoculations, control for the inadvertent increase in relative abundance of other potential pathogens. In our own experimental aquarium facilities, the ciliate type implicated in pathogenesis in this study (JF831359), was prevalent in aquarium samples within one week of initial set up (pers. obs.). Since we show here that these ciliates are absent from non-diseased coral, we strongly recommend that future studies addressing Koch's postulates, simultaneously test for the presence of ciliates via light microscopy and molecular screening to ensure that the stress of applying the inoculate does not inadvertently promote these or other potential pathogens, which are apparently ubiquitous in the field and in experimental aquaria.

Acknowledgements

Thanks to the Deep Aquarium in Hull for tissue samples of corals showing signs of BJS. Funding for this research was provided by the Natural Environmental Research Council, UK (NE/E006949).

References

Ainsworth T., Fine M., Roff G., Hoegh-Guldberg O. (2008) Bacteria are not the primary cause of bleaching in the Mediterranean coral *Oculina patagonica*. *ISME Journal* 2: 67-73.

Ainsworth T.D., Thurber R.V., Gates R.D (2010) The future of coral reefs: a microbial perspective. *Trends in Ecology & Evolution* 25: 233-240.

Ainsworth T.D., Kvennefors E.C., Blackall L.L., Fine M., Hoegh-Guldberg O. (2007) Disease and cell death in white syndrome of Acroporid corals on the Great Barrier Reef. *Marine Biology* 151: 19-29.

Andersen S.B., Vestergaard M.L., Ainsworth T.D., Hoegh-Guldberg O., Kuhl M. (2010) Acute tissue death (white syndrome) affects the microenvironment of tabular *Acropora* corals. *Aquatic Biology* 10: 99-104.

Arboleda M., Reichardt W.G. (2009) Epizoic Communities of Prokaryotes on Healthy and Diseased Scleractinian Corals in Lingayen Gulf, Philippines. *Microbial Ecology* 57: 117-128.

Borneman E.H., Lowrie, J. (2001) Advances in captive husbandry and propagation: An easily utilized reef replenishment means from the private sector? *Bulletin of Marine Science* 69: 897-913.

Bourne D.G., Munn C.B. (2005) Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. *Environmental Microbiology* 7: 1162-1174.

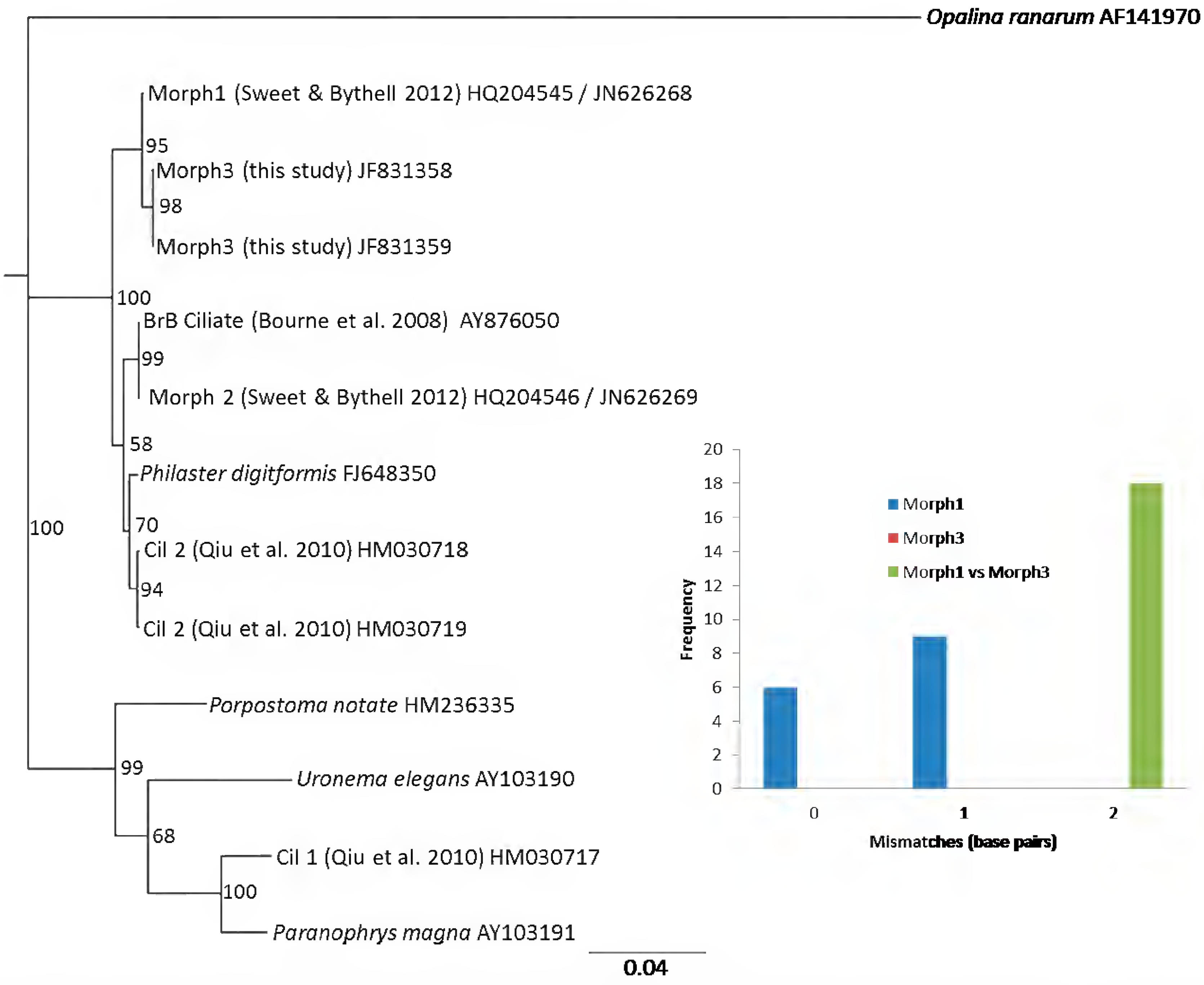
Opalina ranarum AF141970
Morph1 (Sweet & Bythell 2012) HQ204545 / JN626268
95
Morph3 (this study) JF831358
98
Morph3 (this study) JF831359
100
BrB Ciliate (Bourne et al. 2008) AY876050
99
Morph 2 (Sweet & Bythell 2012) HQ204546 / JN626269
58
Philaster digitformis FJ648350
100
70
Cil 2 (Qiu et al. 2010) HM030718
94
Cil 2 (Qiu et al. 2010) HM030719
Porpostoma notate HM236335
99
Uronema elegans AY103190
68
Cil 1 (Qiu et al. 2010) HM030717
100
Paranophrys magna AY103191
0.04
20
18
16
14
12
10
8
6
4
2
0
Frequency
Morph1
Morph3
Morph1 vs Morph3
0
1
2
Mismatches (base pairs)

Figure 6. Neighbour-joining consensus tree of partial 18S rRNA gene sequences of 16 samples of ciliates found within corals exhibiting both brown jelly syndrome and white syndrome within UK aquariums and wild type ciliates found in corals exhibiting signs of white syndrome from Heron Island. Sequences were aligned in Clustal W2, using an IUB cost matrix with a gap open cost of 15 and a gap extend cost of 7. A neighbour-joining consensus tree (1000x re-sampling) was constructed in genius Pro 5.0 using Tamura and Nei (1993) genetic distance model with an opalinid protest, *Opalina ranarium* (AF149070), as the outgroup. Insert histogram shows sequence mismatch frequencies within and between sequences of Morph 1 and 3.

Bruckner A., Bruckner R. (1998) Rapid-wasting disease: Pathogen or predator? *Science* 279: 2023-2025.

Clarke K.R., Warwick R.M. (2001) A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Marine Ecology-Progress Series* 216: 265-278.

Davy S.K., Burchett S.G., Dale A.L., Davies P., Davy J.E., Muncke C., Hoegh-Guldberg O., Wilson W.H. (2006) Viruses: agents of coral disease? *Diseases of Aquatic Organisms* 69: 101-110.

Dopheide A., Lear G., Stott R., Lewis G. (2008) Molecular characterization of ciliate diversity in stream biofilms. *Applied and Environmental Microbiology* 74: 1740-1747.

Fuhrman J.A., Steele J.A., Hewson I., Schwalbach M.S., Brown M.V., Green J.L., Brown J.H. (2008) A latitudinal diversity gradient in planktonic marine bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 105: 7774-7778.

Gil-Agudelo D.L., Fonseca D.P., Weil E., Garzon-Ferreira J., Smith G.W. (2007) Bacterial communities associated with the mucopolysaccharide layers of three coral species affected and unaffected with dark spots disease. *Canadian Journal of Microbiology* 53: 465-471.

Guppy R., Bythell J.C .(2006) Environmental effects on bacterial diversity in the surface mucus layer of the reef coral Montastraea faveolata. *Marine Ecology-Progress Series* 328:133-142

Hormansdorfer S., Wentges H., Neugebaur-Buchler K., Bauer J. (2000) Isolation of Vibrio alginolyticus from seawater aquaria. *International Journal of Hygiene and Environmental Health* 203: 169-175.

Hummon W. (2008) *Meiofauna Marina. Biodiversity, morphology and ecology of small benthic organisms*. Department of Biological Sciences, Ohio University, USA

Janse I., Bok J., Zwart G. (2004) A simple remedy against artifactual double bands in denaturing gradient gel electrophoresis. *Journal of Microbiological* Methods 57: 279-281.

Jousset A., Lara E., Nikolausz M., Harms H., Chatzinotas A. (2010) Application of the denaturing gradient gel electrophoresis (DGGE) technique as an efficient diagnostic tool for ciliate communities in soil. *Science of the Total Environment* 408:1221-1225

Klaus JS., Frias-Lopez J., Bonheyo G.T., Heikoop J.M., Fouke B.W. (2005) Bacterial communities inhabiting the healthy tissues of two Caribbean reef corals: interspecific and spatial variation. Coral Reefs 24: 129-137.

Kvennefors E.C.E., Sampayo E., Ridgway T., Barnes A.C., Hoegh-Guldberg O. (2010) Bacterial communities of two ubiquitous Great Barrier Reef corals reveals both site and species-specificity of common bacterial associates. *PLoS One* 5: e10401.

Lecampionalsumard T., Golubic S., Priess K .(1995) Fungi in Corals - Symbiosis or Disease - Interaction between Polyps and Fungi Causes Pearl-Like Skeleton Biomineralization. *Marine Ecology-Progress* Series 117: 137-147.

Lesser M.P., Bythell J.C., Gates R.D., Johnstone R.W., Hoegh-Guldberg O. (2007) Are infectious diseases really killing corals? Alternative interpretations of the experimental and ecological data. *Journal of Experimental Marine Biology and Ecology* 346: 36-44.

Lobban C.S., Raymundo L., Montagnes D.J.S. (2011) *Porpostoma guamensis n. sp.*, a Philasterine Scuticociliate Associated With Brown-Band Disease of Corals. *Journal of Eukaryotic Microbiology* 58: 103-113.

Luna G.M., Biavasco F., Danovaro R. (2007) Bacteria associated with the rapid tissue necrosis of stony corals. *Environmental Microbiology* 9:1851-1857

Luna G.M., Bongiorni L., Gili C., Biavasco F., Danovaro R. (2010) *Vibrio harveyi* as a causative agent of the white syndrome in tropical stony corals. *Environmental Microbiology Reports* 2: 120-127.

Marhaver K.L., Edwards R.A., Rohwer F. (2008) Viral communities associated with healthy and bleaching corals. *Environmental Microbiology* 10: 2277-2286.

Muyzer G., Smalla K. (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 73: 127-141.

Oldach D.W., Delwiche C.F., Jakobsen K.S., Tengs T., Brown E.G., Kempton J.W., Schaefer E.F., Bowers H.A., Glasgow H.B., Burkholder J.M., Steidinger K.A., Rublee P.A. (2000) Heteroduplex mobility assay-guided sequence discovery: Elucidation of the small subunit (18S) rDNA sequences of *Pfiesteria piscicida* and related dinoflagellates from complex algal culture and environmental sample DNA pools. *Proceedings of the National Academy of Sciences of the United States of* America 97: 4303-4308.

Qiu D.J., Huang L.M., Huang H., Yang J.H., and Lin S.J. (2010) Two functionally distinct ciliates dwelling in *Acropora* corals in the South China Sea near Sanya, Hainan Province, China. *Applied Environmental Microbiology* 76: 5639–5643.

Sanchez O., Gasol JM., Massana R., Mas J., Pedros-Alio C. (2007) Comparison of different denaturing gradient gel electrophoresis primer sets for the study of marine bacterioplankton communities. *Applied and Environmental Microbiology* 73: 5962-5967.

Selinummi J., Seppala J., Yli-Harja O., Puhakka JA.. (2005) Software for quantification of labeled bacteria from digital microscope images by automated image analysis. *Biotechniques* 39: 859-863.

Siboni N., Lidor M., Kramarsky-Winter E., Kushmaro A. (2007) Conditioning film and initial biofilm formation on ceramics tiles in the marine environment. *FEMS Microbiology Letters* 274: 24-29.

Song W.B. (2000) Morphological and taxonomical studies on some marine scuticociliates from China Sea, with description of two new species, *Philasterides armatalis sp n*, and *Cyclidium varibonneti sp n.* (Protozoa: Ciliophora: Scuticociliatida). *Acta Protozoologica* 39: 295-322.

Sussman M., Willis B.L., Victor S., Bourne D.G. (2008) Coral Pathogens Identified for white syndrome (WS) Epizootics in the Indo-Pacific. *PLoS One* 3

Sweet M.J., Bythell J.C. (2012) Ciliate and bacterial communities associated with white syndrome and Brown Band Disease in reef building corals. *Environ Microbiol* 14: 2184-2199.

Sweet M.J., Croquer A., Bythell J.C. (2010) Temporal and spatial patterns in waterborne bacterial communities of an island reef system. *Aquatic Microbial Ecology* 61: 1-11.

Sweet M.J., Jones R., Bythell J.C. (2011a) Coral diseases in aquaria and in nature. *Journal of the Marine Biological Association of the United Kingdom* 92: 791-801.

Sweet M.J., Croquer A., Bythell J.C. (2011b) Dynamics of bacterial community development in the reef coral *Acropora muricata* following experimental antibiotic treatment. *Coral Reefs* 30: 1121-1133.

Thompson J.R., Randa M.A., Marcelino L.A., Tomita-Mitchell A., Lim E., Polz M.F. (2004) Diversity and dynamics of a north Atlantic coastal Vibrio community. *Applied and Environmental Microbiology* 70:4103-4110

Vincent A.C.J. (2006) Live food and non-food fisheries on coral reefs, and their potential management. In: Cote IMRJD (ed) *Coral Reef Conservation*, pp. 183-236

Wabnitz C., Taylor M., Green E., Razak T. (2003) *From ocean to aquarium. The global trade in marine ornamental species*.

Walsh P.S., Metzger D.A., Higuchi R. (1991) Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from rorensic material. *Biotechniques* 10: 506-513.

Weinbauer M.G., Beckmann C., Hofle M.G. (1998) Utility of green fluorescent nucleic acid dyes and aluminum oxide membrane filters for rapid epifluorescence enumeration of soil and sediment bacteria. *Applied and Environmental Microbiology* 64: 5000-5003.

Willis B.L., Page C.A., Dinsdale E.A. (2004) Coral disease on the Great Barrier Reef. In: Rosenberg E., Loya Y (eds) *Coral health and disease*. Springer-Verlag Heidelberg pp. 69-104.

Work TM., Aeby GS (2011) Pathology of tissue loss (white syndrome) in *Acropora* sp corals from the Central Pacific. *Journal of Invertebrate Pathology* 107: 127-131.

Yamaguchi N., Baba T., Nakagawa S., Saito A., Nasu M. (2007) Rapid monitoring of bacteria in dialysis fluids by fluorescent vital staining and microcolony methods. *Nephrology Dialysis Transplantation* 22: 612-616.

Yu W., Dodds W.K., Banks M.K., Skalsky J., Strauss E.A. (1995) Optimal Staining and Sample Storage Time for Direct Microscopic Enumeration of Total and Active Bacteria in Soil with 2 Fluorescent Dyes. *Applied and Environmental Microbiology* 61: .3367-3372

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Research article

Investigations on rumen health of different wild ruminants in relation to feeding management

Barbara Schilcher[1,2,*], **Katrin Baumgartner**[2], **Hans Geyer**[3], **Annette Liesegang**[1]

[1]Department of Animal Nutrition, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, CH-8057 Zurich, Switzerland
[2]Tiergarten Nuremberg, Am Tiergarten 30, D-90480 Nuremberg, Germany
[3]Department of Veterinary Anatomy, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, CH-8057 Zurich, Switzerland
**Corresponding author: Barbara.Schilcher@gmx.de*

Keywords:
captive wild ruminants, rumen epithelium, subacute ruminal acidosis, diet composition

Article history:
Received: 06 December 2012
Accepted: 28 February 2012
Published online: 24 July 2013

Abstract

In this study, the rumen metabolism of four ruminant species – blackbuck (*Antilope cervicapra*), barbary sheep (*Ammotragus lervia),* sika deer (*Cervus nippon dybowskii*) and addax antelope (*Addax nasomaculatus*) – from the Nuremberg Zoo was investigated in relation to their feeding management. Parameters of microbial fermentation in the rumen as well as rumen tissue samples were examined. Additionally, the offered and refused food was weighed, analysed and a dietary intake calculation for these ruminant species was performed. Three of the four ruminant species – blackbuck, barbary sheep and addax antelope – all grazers, showed severe characteristics of subacute ruminal acidosis due to a diet high in fermentable carbohydrates (NfE ≥ 55% of DM basis) and low in fibre (XF ≤ 18% of DM basis). This was indicated by numerous microabscesses within the rumen mucosa. These results demonstrate that subacute ruminal acidosis is still a severe problem in captive wild ruminants, which are often fed inappropriate diets with a high concentration of easily digestible carbohydrates.

Introduction

Ruminants represent a group of highly sophisticated herbivores, which are often specialized for particular groups of feeding plants and cannot be fed with uniform rations (Hofmann 1995). Therefore Hofmann and Stewart (1972) classified them into a flexible system of three overlapping morphophysiological feeding types: concentrate selectors, grass and roughage eaters and intermediate, opportunistic mixed feeders. Today “grass- and roughage eaters” and particularly the contradictory term “concentrate selectors” are replaced by the terms “grazers” and “browsers” according to the natural forage of these ruminants (Clauss *et al.* 2003). Numerous studies, for example Ippen and Henne (1985; 1988), Kiupel (1988) and Hatt *et al.* (1995), document the high incidence of gastrointestinal disorders in zoo ruminants due to an inadequate feeding regime. One of the most prominent examples for gastrointestinal disorders in ruminants is ruminal acidosis. This was investigated in detail by Marholdt (1991), who focused on pathological changes of the rumen mucosa of 67 captive wild ruminants. 40% of these investigated animals showed acidotic characters within the rumen mucosa.

In the past few years several indications for subacute ruminal acidosis in certain ruminant species of Nuremberg Zoo were noticed: For example, deformed claws in the barbary sheep (*Ammotragus lervia*), although the claws were regularly trimmed, and poor body condition plus episodes of diarrhoea in the addax antelopes (*Addax nasomaculatus*). The purpose of the study was to investigate the rumen health and feeding management of different ruminant species – blackbuck (*Antilope cervicapra*), barbary sheep (*Ammotragus lervia*), sika deer (*Cervus nippon dybowskii*), addax antelope (*Addax nasomaculatus*) - from the Nuremberg Zoo.

Methods

Five individuals of each ruminant species mentioned above were used in this study. All animals were included opportunistically having been selected for removal due to to problems in group composition and overcrowding, or in two cases in the blackbuck group due to an acute injury. The sample collection from these animals begun immediately after death and was completed within 30 minutes.

Samples of rumen fluid were taken immediately for measuring the pH with a portable electronic pH meter (SM 102®, Milwaukee, USA). Further rumen fluid was centrifuged (4 min at 6000 rpm) and stored with perchlor acid (0.66N) respectively mercurouse chloride (1.25%) for measuring lactic acid (UV-test with Cobas Mira Roche-Autoanalyser®, F. Hoffmann-La Roche Ltd., Basel, Switzerland) respectively short chain fatty acids (SCFA) by gas chromatography (Tangermann and Nagengast 1996).

Furthermore tissue samples of the rumen atrium were taken (1 cm x 2 cm pieces). After fixation in neutral buffered formaldehyde (4%), dehydration in graded ethanol and embedding in paraffin transverse sections of 5 mm thickness were prepared and stained with hematoxylin and eosin to verify the various epithelial layers in the rumen mucosa. The histological evaluation of the rumen mucosa according to the method of Marholdt (1991) where a standardisation of changes caused by acidosis in comparison with normal rumen mucosa, was performed. Additionally, on every microscope slide the number of rumen papillae and micro-abscess was evaluated and the relation of micro-abscess to papillae was calculated.

The diets (roughage, concentrates and fresh food) offered to the animals of the four ruminant species were listed and weighed as well as the refused food. A proximate analysis for dry matter (DM), ash (XA), crude fibre (XF), and crude protein

(N*6.25) (XP) was carried out by standardized procedures. Data for crude fat (XL) was taken from the literature (Kamphues *et al.* 2009, Souci *et al.* 2008, FAG 1994) respectively the official declaration of the pelleted compound food. Nitrogen free extracts (NfE) were calculated as NfE = 100-(XA+XL+XF+XP). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were estimated by sequential fibre analyses (Van Soest 1967). For each ruminant species the ingested diet composition was calculated.

Data are presented as mean ± standard deviation (SD) for each ruminant species. A one-way analysis of variance (ANOVA) was performed with the factor "group". This was followed by the one way Kruskal-Wallis test. The analyses were calculated by use of a statistical software program (Systat for Windows® 11.0, SPSS Inc. Chicago, USA) For all tests the level of significance was set at $p = 0.05$, so if the p-value was less than 0.05, differences were considered statistically significant.

Results

Parameters of rumen fermentation

The mean rumen pH of all investigated species was between 6.3 and 6.6 (Table 1). There was a significant difference ($p < 0.05$) between the mean rumen pH of sika deer and addax antelope.

Neither d-lactate nor l-lactate were detectable in the rumen fluid of any animal (detection limit: 0.3 mg/l). The SCFA concentrations in the rumen fluid of the four investigated species were between 50 and 81 mmol/l (Table 1)

Species	BW (kg)	pH	SCFA (mmol/l)
Antilope cervicapra	27 ± 8	6.3 ± 0.4	71 ± 20
Ammotragus lervia	64 ± 42	6.5 ± 0.4	81 ± 29
Addax nasomaculatus	69 ± 28	6.3 ± 0.2*	80 ± 13
Cervus nippon dybowskii	69 ± 11	6.6 ± 0.2*	50 ± 13

Table 1. Mean body weight (BW) ± standard deviation (SD) of the invesigated animals and mean pH and concentration of short chain fatty acids (SCFA) ± SD in the rumen fluid. * $p<0.05$

Rumen epithelium

The rumen mucosa of all five investigated sika deer showed a multilayered epithelium. Particularly the Stratum corneum with ballooned and low keratinised cells indicates active resorption due to a large amount of easily digestible carbohydrates. In contrast, severe characteristics of ruminitis were present in the rumen mucosa of blackbuck, barbary sheep and addax antelopes. There were numerous aggregations of leukocytes, interpreted as micro abscesses (Figure 1), as well as diffuse spread leukocytes within the rumen epithelium in these species. The ratio of micro abscesses to the number of papillae on one slice as well as the presence of diffuse spread leukocytes in the rumen mucosa are shown in Table 2. All except one of the five investigated blackbuck (BB 1), all the investigated barbary sheep (BS 1-5) and all addax antelopes (AA 1-5) had an abnormal Stratum corneum consisting of several layers of flat epithelial cells containing high concentrations of keratin.

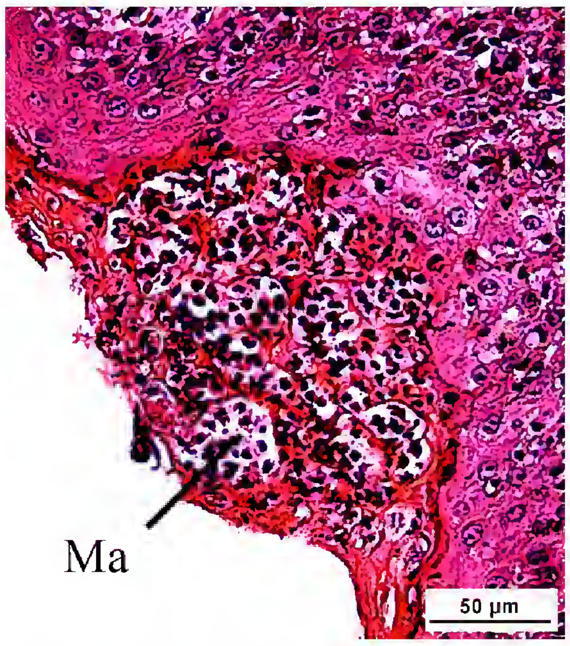

Figure 1: Rumen epithelium of a barbary sheep (BS 4) with cumulation of leukozytes, interpreted as a micro abscess (Ma).

Ingested diet composition

Animals	Ma / papillae	Diffuse spreaded leukocytes
BB 1	-	No
BB 2	0.03	No
BB 3	-	Yes
BB 4	-	Yes
BB 5	-	No
BS 1	1.42	Yes
BS 2	0.15	Yes
BS 3	0.57	Yes
BS 4	1.14	Yes
BS 5	0.16	
AA 1	0.25	Yes
AA 2	0.79	Yes
AA 3	0.29	Yes
AA 4	-	No
AA 5	-	Yes

Table 2. *Ratio of micro abscesses (Ma) in the rumen mucosa to rumen papillae on one microscopcal slice and the presence of diffuse spreaded leukocytes in the rumen mucosa of the investigated grazer species. BB = Blackbuck; BS = Barbary sheep; AA = Addax antelope.*

Species	Roughage	Concentrates	Vegetables/ Fruits	R:C:V/F in % on DMB
Antilope cervicapra	Hay	Savannenfutter®* Kofu®* Oats Bran	Apples Carrots Beets	16:77: 7
Ammotragus lervia	Hay	Ziegenpellets®* Kofu® Bran Oat flakes	Beets	37:49:14
Addax nasomaculatus	Hay	Kofu® Oat flakes	Apples Carrots	39:57: 4
Cervus nippon dybowskii	Hay	Kofu® Corn pellets Oats Bran	Beets	18:57:25

Table 3. Ingested diet composition and the ratio of roughage (R) to concentrates (C) to vegetables and fruits (V/F) on dry matter basis (DMB) in the ingested diets. * Savannenfutter®, Kofu® and Ziegenpellets® are pelleted compound feeds special compounded for the Nuremberg Zoo.

Species	DM %	XP %	XF %	XL %	NfE %	NDF %	ADF %	ADL %
Antilope cervicapra	63	17	14	3	58	40	18	4
Ammotragus lervia	50	14	18	3	55	44	23	4
Addax nasomaculatus	74	16	18	3	55	44	22	4
Cervus nippon dybowskii	37	11	15	3	65	38	20	4

Table 4. Proximate analysis of the ingested diets (in winter) of the four ruminant species; DM = dry matter, XP = crude protein, XF = crude fibre, XL = crude fat, NfE = nitrogen free extracts, NDF = Neutral detergent fibre, ADF = Acid detergent fibre, ADL = Acid detergent lignin. XP, XF, XL, NfE, NDF, ADF and ADL are given in % of dry matter basis.

The composition of the ingested diets of the four ruminant species and the ratio of roughage to concentrates in the winter diets are listed in Table 3. In summer all species additionally get grass but no beets. The addax antelopes were separated indoor over night and were also fed separately in their boxes. In contrast there was a group feeding situation with the blackbuck, barbary sheep and sika deer. The animals of the investigated ruminant species were fed with the concentrates, vegetables and fruits once daily but hay was available all day ad libitum. The proximate analysis of the chemical components of the ingested diets are shown in Table 4.

Discussion

The parameters of rumen fluid (pH, SCFA, lactate) are closely related with feeding regime (Steger *et al.* 1970). In this study these values must be interpreted with caution because due to zoo management reasons (opening times for visitors), the sample collection had to be done early in the morning before the animals were fed. Therefore samples were taken long after the last meal when the pH is upregulated and SCFA concentrations lowest. This would explain why the parameters like pH and SCFA concentration, which are usually changed during subacute acidosis are not changed in the present study. After the single daily feeding of concentrates, vegetables and fruits however, the pH is decreased which leads to lesions in the long term. The concentration of lactate in the rumen fluid is decreased during acute ruminal acidosis but not during subacute ruminal acidosis (Nocek 1997).

The severe lesions of the rumen mucosa of the blackbucks, barbary sheep and addax antelopes are fundamental characteristics of subacute ruminal acidosis (Krause and Oetzel 2006). The reasons for developing subacute ruminal acidosis in these cases were probably the diets, which consisted of high amounts of concentrates and fresh food. A high level of NfE (55-65% of DM basis) and a low level of NDF (38-44% of DM basis) combined with high amounts of concentrates, fruits and vegetables leads to the assumption that a big part of the NfE-fraction consisted of starch and sugars. Additionally, farmed fruits like apples have a very fast fermenting time and lead to a fast production of high amounts of SCFA (Hummel *et al.* 2006). Furthermore, all four diets had a deficiency of fibre since crude fibre contents of the diets (14-18% of DM basis) reached the critical threshold (18%) of beef cattle (Kamphues *et al.* 2009). Diets with high amounts of easily digestible carbohydrates combined with a deficiency of fibre causes a temporarily altered state of the rumen (low pH after feeding) which may have lead in the cases of blackbuck, barbary sheep and addax antelopes to the lesions of rumen epithelium. Additionally the feeding regime, like the number of meals and the sequence of roughage and concentrates during feeding is important to prevent subacute ruminal acidosis (Nordlund *et al.* 1995). In the case of Nuremberg Zoo the investigated animals were usually fed once a day, normally in the morning, with a mixture of concentrates, vegetables and fruits. At this time hay also was offered in amounts which passed for the whole day. The problem with this feeding situation is that the animals usually eat the concentrates, vegetables and fruits in preference so hay intake is initially low. This might have influenced the rumen milieu in a negative way, since synchronity (the synchronic ingestion of protein and energy, which is important for the ruminant microbial flora), was not guaranteed with this feeding regime.

In the group of sika deer there were no indications of subacute ruminal acidosis since there were no pathological changes in the ruminal mucosa. Furthermore in this species the rumen pH was highest (6.6) and the concentration of SCFA lowest (50 mmol/l). This could lead to the conclusion that the sika deer, classified as an intermediate feeder, seemed to tolerate increased amounts of highly fermentable carbohydrates since no pathological changes were present. This is in contrast to the findings of Kuipel (1988), Marholdt (1991), and Clauss *et al.* (2003) who showed that intermediate feeders and browsers are even more prone than grazers to GIT disorders, like subacute ruminal acidosis, due to a high amount of concentrates in the diets. Apart from the classification in different feeding-types the age of the investigated animals could be of relevance in this case. Whereas the investigated groups of blackbuck, barbary sheep and addax antelopes were heterogenous (age ranged from 8 month to 15 years) in the group of sika deer the animals were aged between 12 and 20 months. Additionally, younger animals are normally more subdominant and consequently were not able to get high amounts of concentrates in a group feeding situation compared to the dominant animals. Apart from the diet with high amounts of concentrates the sika deer used in this study were probably too young to develop long term effects in the rumen mucosa.

Conclusions

The blackbuck, barbary sheep and addax antelopes, all grazers, showed severe characteristics of subacute ruminal acidosis due to a diet high in fermentable carbohydrates and low in fibre.

The feeding management, the ingested diet composition and the feeding regime (frequency and sequence), of those ruminant species from Nuremberg Zoo must be improved. The diets should consist mainly of roughage (hay of good quality) and only that amount of low energy pelleted compound food to cover specific demands on vitamins and minerals like vitamin E and selenium. Fresh food such as apples and carrots should not be fed since they are not adequate for a physiological rumen milieu even in a grazing ruminant species. The single daily meal of concentrates should be divided at least into two meals a day. Changing the sequence of food provision so that some roughage is consumed before the concentrate meal, particularly in the morning, would also be very important.

Acknowledgements

We would like to thank the staff of Nuremberg Zoo for their help and friendly support as well as the laboratory assistances of the Institute of Animal Nutrition, Vetsuisse Faculty, for their assistance with sample analysing.

References

Clauss M., Kienzle E., Hatt J.M. (2003) Feeding practice in captive wild ruminants. Peculiarities in the nutrition of browsers / concentrate selectors and intermediate feeders. A review. In Fidgett A., Clauss M., Ganslosser U., Hatt J.M., Nijboer J. (eds) *Zoo animal nutrition* Vol. II. Fuerth, Germany: Filander.

FAG (Eidgenössische Forschungsanstalt für Viehwirtschaftliche Produktion, Posieux, Schweiz) (1994) *Fütterungsempfehlungen und Nährwerttabellen für Wiederkäuer.* 3rd edition. Zollikofen, Switzerland: Landwirtschaftliche Lehrmittelzentrale.

Hatt J-M., Hauser B., Baumgartner R., Isenbügel E. (1995) Sektionsergebnisse bei Wiederkäuern im Zoo Zürich – Eine Auswertung unter spezieller Berücksichtigung fütterungsbedingter Erkrankungen. *Verh. Ber. Erkrg. Zootiere* 37: 259-265.

Hofmann R.R., Stewart D.R.M. (1972) Grazer or browser: a classification based on the stomach structure and feeding habits of east African ruminants. *Mammalia* 36: 226-240.

Hofmann R.R. (1995) Nahrungsauswahl und Spezialisierung des Verdauungsapparates bei Wildwiederkäuern – Ein Haltungsproblem für Zootiere. *Verh. Ber. Erkrg. Zootiere* 37:233-236.

Hummel J., Hovenjürgen M., Niess E., Johansen K., Nijboer J., Zimmermann W. (2006) Considerations on feed used for zoo ruminants. In Fidgett A..Clauss M., Eulenberger K., Hatt J.M, Hume I., Janssens G., Nijboer J. (eds) *Zoo animal nutrition* Vol. III. Fuerth, Germany: Filander.

Ippen R., Henne D. (1985) Ein Beitrag zu den Erkrankungen der Cerviden. *Verh. Ber. Erkrg. Zootiere* 27: 7-16.

Ippen R., Henne D. (1988) Auswertung der Obduktionsbefunde bei 2000 Artiodactyla. *Verh. Ber. Erkrg. Zootiere* 30: 5-24.

Kamphues J., Coenen M., Iben C., Kienzle E., Pallauf J., Simon O., Wanner M., Zentek J. (2009) *Supplemente zur Vorlesung und Übungen in der Tierernährung.* Hannover, Germany: M & H Schaper GmbH.

Kiupel H. (1988) Sektionsergebnisse bei Paarhufern (Artiodactyla). *Verh. Ber. Erkrg. Zootiere* 30:29-34

Krause K.M., Oetzel G.R. (2006) Understanding and preventing ruminal acidosis in dairy herds: a review. *Anim. Feed. Sci. Technol.* 126:215-236

Marholdt F. (1991) *Fütterungsbedingte, morphologische Veränderungen der Vormagenschleimhaut von 67 Zoo-Wiederkäuern im Vergleich mit wildlebenden Wiederkäuern.* Dissertation. Giesse, Germany: Universitiy of Giessen

Nocek J.E. (1997) Bovine acidosis: implications on laminitis. *J. Dairy Sci.* 80: 1005-1028.

Nordlund K.V., Garrett E.F., Oetzel G.R. (1995): *Herd-based rumenocentesis: a clinical approach to the diagnosis of subacute rumen acidosis. The Compendium X.* pp. 48-56.

Souci S.W., Fachmann W., Kraut H. (2008) *Food composition and nutrition tables – Die Zusammensetzung der Lebensmittel, Nährwerttabellen – La composition des aliments, Tableuax des valeurs nutritives.* 7th edition. Stuttgart: Wissenschaftliche Verlagsgesellschaft, Medpharm Taylor & Francis.

Steger H., Voigt J., Piatkowski B. (1970) Einfluss der Futterration und des Entnahmezeitpunktes von Pansensaft auf seine Zusammensetzung in Untersuchungen an Kühen. *Arch. Tierernährung* 20: 349-359.

Tangermann A., Nagengast F.N. (1996) A gas chromatographic analysis of fecal short chain fatty acids, using the direct injection method. *Analytical Biochemistry* 236: 1-8.

Van Soest P.J. (1967) Development of a comprehensive system of feed analysis and its application to forages. *J. Anim. Sci.* 26: 119-125.

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Research Article

The influence of zoo visitor numbers on the behaviour of harbour seals (*Phoca vitulina*)

Jeroen M.G. Stevens[a,*], **Anya Thyssen**[b], **Hans Laevens**[b], **Hilde Vervaecke**[b]

[a] Centre for Research and Conservation, Royal Zoological Society of Antwerp. Koningin Astridplein 26, B-2018 Antwerpen, Belgium
[b] KaHoSL Sint Lieven, Hospitaalstraat 23-26, B-9100 Sint Niklaas, Belgium
**Corresponding author: Jeroen.Stevens@kmda.org*

Keywords: seal; visitor effect; behaviour; captivity; zoo

Article history
Received: 13 April 2012
Accepted: 16 July 2013
Published online: 24 July 2013

Abstract

We investigated the influence of zoo visitor numbers on the behaviour of a group of eight harbour seals at Antwerp Zoo. The behaviour of the seals was monitored using instantaneous scan sampling. Visitor presence at the enclosure was also monitored instantaneously. Additionally, daily visitor numbers for the zoo were gathered. We related seal behaviour to both the number of visitors present during the instantaneous sampling and to the daily visitor attendance. Both analyses showed that under increasing visitor numbers, more seals submerged under water. While behavioural changes are clear and it appears the seals were hiding from increasing visitor numbers by diving under water, it remains hard to assess whether visitors compromise the welfare of seals in captivity

Introduction

Since the 1970s, several studies have shown that the presence, number and behaviour of zoo visitors can all influence the behaviour of the animals (reviews in Hosey 2000; Davey 2007). The presence of visitors can influence animal behaviour in zoos negatively (Chamove *et al.* 1988; Davis *et al.* 2005; Hosey, 2000) or positively (Cook and Hosey 2005). However results are somewhat ambiguous (Hosey 2000) due to several confounding factors, such as animal size, visitor behaviour and enclosure design (Davey 2007; Ross *et al.* 2007). Most studies into visitor effects have been done on primates (e.g. Hosey and Druck 1987: Mitchell et al., 1992; Wells, 2005) ungulates (Thompson, 1989) and carnivores (Margulis *et al.* 2003). Within the primate group there is a general trend for negative effects (Hosey 2000). For other animal groups, data are scant and more research in more different groups of animals is needed before conclusions on the relationship between visitor numbers and animal welfare can be made (Hosey 2000; Davey 2007).

One of the methodological considerations in visitor studies is the discrepancy between instantaneous evaluation and daily evaluation (Kuhar 2008). Instantaneous evaluations are usually based on interval sampling (Martin and Bateson 1993) throughout the day to score animal behaviour and visitor characteristics. Thus crowd size at the animal enclosure is accurately measured and can vary throughout the day. However, successive intervals are not independent of each other, cumulative effects of varying visitor numbers cannot be measured and the behaviour of the animals can influence visitor numbers, so that causality is hard to detect (Kuhar 2008). It has been pointed out that more active animals will attract more visitors, and thus increased visitor density may be a consequence rather than a cause of changes in animal behaviour (Hosey 2000). This has been labelled the "visitor effect/visitor attraction confound", which can influence the results of instantaneous evaluations (Kuhar 2008, p. 378; Hosey 2000).

In daily evaluation, the number of visitors entering the park or passing in front of the enclosure is used as the independent variable in the analysis, which takes into account cumulative effects and can be used to avoid the visitor-effect/visitor-attraction confound (Kuhar 2008). However, no detailed information on interactions between visitors and animals is gathered in this method. Therefore, a combination of both methods seems useful to study the impact of zoo visitors on animal behaviour.

Seals seem likely to be influenced by visitors. Studies on different species of seals in the wild show effects of human presence on seal behaviour. Harp seal (*Phoca groenlandicus*) mothers and pups showed increased alertness and rested less when tourists were present (Kovacs and Inness 1990). Cassini (2001) reports that wild fur seals (*Arctocephalus australis*) retreat when tourists approach them closer than 10 meters. So in wild seals there seems to be a clear threshold distance. On the other hand, Wedell seals (*Leptonychotes weddellii*) habituated very easily to repeated pedestrian approaches, resulting in less looking up at approaching humans (Van Polanen Petel *et al.* 2008). In zoos, seals are a highly popular species for the public and some preliminary studies indicate that a visitor effect exists. Taylor *et al.* (1988) report that seals show increased vigilance behaviour when confronted with an unfamiliar observer, but they were quickly habituated to a familiar observer. Morgan and Tromborg (2007) cite unpublished data that show increased vigilance in harbour seals with increasing visitor numbers and noise. The authors do not indicate whether visual scanning is a sign of negative or positive visitor effects.

Our aim is to use a combination of instantaneous evaluation and daily evaluation to investigate to what extent zoo-born harbour seals are habituated to the presence of crowds of zoo visitors and whether a positive or negative visitor effect exists for this species.

Methods

Study group and enclosure

We studied a group of eight harbour seals, four males and four females, at Antwerp Zoo, Belgium. All seals were captive born and six of them were born in Antwerp Zoo. The seals were housed in a 162 m² kidney shaped pool with a beach. In the enclosure, balls barrels and platforms were provided as environmental enrichment. The backside of the enclosure was formed by a high wall of artificial rockwork. The visitors only had access to the front of the enclosure, alongside the pool and alongside a small part of the beach. On average, visitors were standing about one to 1.5m above the water level and so looked down on the seals. There were no further barriers between the seals and visitors that would create any additional distance; apart from a small patch of shrubs alongside half of the beach.

Observation protocol and ethogram

The second author observed the seal group on 24 weekdays over a period of six weeks between February and April 2008. No observations were made during the weekends. Each day, two to three observation sessions of ten minutes each were carried out semi-randomly throughout the day between 10.00h and 17:00h, with a minimum interval of 60 minutes between two subsequent observations on the same day (Margulis and Westhus 2008). In this way, a total of 61 observation sessions, totalling ten observation hours, was carried out. Within an observation session, we used instantaneous scan sampling (Altmann 1974) with 30-second intervals. The seals were difficult to individually distinguish, especially when swimming under water. Therefore we did not note individual identities, but simply recorded the number of animals performing specific behaviours in each sample. On each scan we scored a) how many visitors were standing alongside the seal pool b) how many seals were in each of the following behavioural categories (after Hunter *et al.* 2002). The behavioural categories were identical for both instantaneous and daily analyses, and were defined as follows:

1. Swimming under water: body and head of the seal are totally submerged in the water
2. Swimming with head above water: seal actively swims with head (partly) above the water
3. Visually scanning the environment: seal hangs vertically in the water with head above the water, clearly scanning the environment by looking around.
4. Resting (partially) on land: seal lies on the beach area of the enclosure (Hunter *et al.* 2002: "resting hauled out")
5. Social interactions: seal engages in social activities including categories of breeding behaviour and aggressive behaviour described in Hunter *et al.* (2002)

Only visitors that were actually standing still, with two feet on the ground and visually oriented towards the seals, were counted; i.e. visitors walking past the enclosure were not counted as present. The observer was never counted as a visitor.

Statistical analyses

We investigated the influence of daily visitor attendance on seal behaviour and the instantaneous effect in two separate Multinomial Regression Models in SAS (vs9.0). For the daily evaluation, we obtained the daily number of visitors in the zoo from the ticket office. We chose the seven days with the highest number of visitors ("high visitor attendance" mean number of visitors per day= 3756 ± 464.2) and compared this sample with the seven days of the lowest visitor attendance ("low visitor attendance" mean number of visitors per day = 669.5 ± 48.0 visitors). The other 10 days were left out of these analyses. For each of the 14 sample days, two full observations (with 21 scans each) were chosen. We used the proportion of seals recorded in each behavioural category as dependent variable and 2 independent variables: the test condition (small crowds versus large crowds) as well as maximum daily temperature.

For the instantaneous evaluation, we performed a Multinomial regression model on the full dataset of 1279 scan samples and for each sample noted how many visitors were present at the enclosure (continuous independent variable) , and the proportion of seals scored in each behavioural category as a dependent category. Temperature was not considered for the instantaneous evaluation as it was not measured at each individual sample. After the multinomial regression we did separate regression models for each behavioural category, to look for more specific effects.

Results

Daily evaluation – visitor attendance and seal behaviour

When comparing maximum daily temperatures for low visitor attendance and high visitor attendance, we found a non-significant difference between the two periods (t-test: t = 2.01; p = 0.07). When we compared days of high visitor attendance versus low visitor attendance, we found that the behaviour of the seals was significantly influenced by condition (χ^2 = 18.21, df = 1, p < 0.0001; Fig 1.) but not temperature (χ^2 = 0.39, df=1, p=0.53). Post hoc tests for each behavioural category showed that more seals spent time under water at high visitor attendance compared to low visitor attendance (χ^2 = 11.37; p = 0.0007). On the other hand, on high visitor attendance days, significantly fewer seals were scanning the environment (χ^2 = 28.92 p < 0.0001) and resting on land (χ^2 = 91.69 p < 0.0001). We found no significant differences between the two conditions in the number of seals swimming with their heads above the water (χ^2 = 0.66; p = 0.23) or engaged in social behaviour (χ^2 = 3.23, p= 0.07).

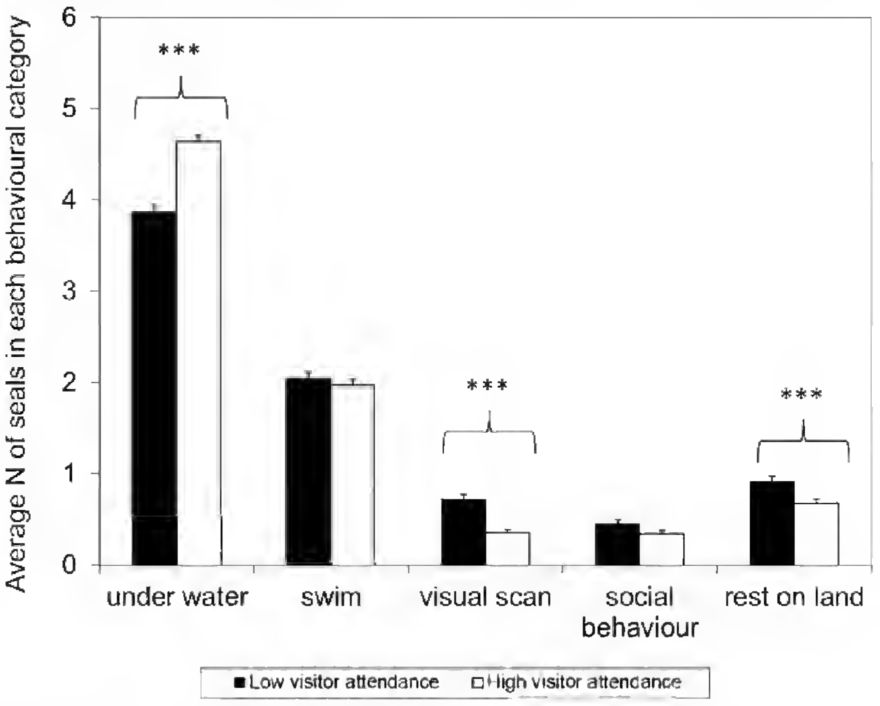

Figure 1. Average number of seals in each behavioural categories on low visitor attendance days (black: < 853 visitors in zoo) and on high visitor attendance days (white: > 1800 visitors in zoo) + Standard error of means. Triple asterisks indicate significant differences p< 0.001

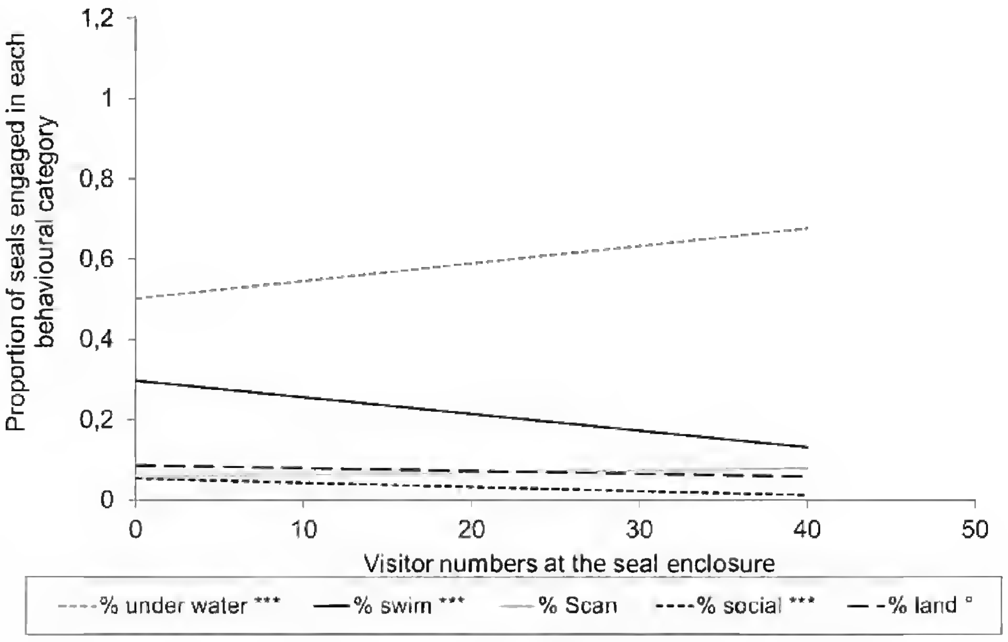

Figure 2. Instantaneous evaluation of seal behaviour in relation to number of visitors standing in front of the enclosure. Triple asterisks indicate significant differences p< 0.001; ° indicates a trend with 0.05 < p < 0.10

Instantaneous evaluation - Visitor presence and seal behaviour

Overall, we found that the absolute number of visitors standing around the enclosure had a significant effect on the behaviour of the seals ($F_{1,1253}$ = 59.30, $p < 0.0001$, Fig 2). The analyses per behaviour separately showed that as the visitor numbers increased, more seals were seen swimming underwater (χ^2 = 22.92; $p < 0.0001$), fewer seals were seen swimming with heads above the water (χ^2 = 40,95; $p< 0.001$), fewer seals were seen in social behaviour (χ^2 = 14.66, $p < 0.0001$). There was only a statistical trend that showed fewer seals resting on land (χ^2 = 3.74; $p = 0.053$), and no significant effect of visitor numbers on the number of seals scanning the environment (χ^2 = 2.58; $p = 0.11$).

Discussion

We used both daily evaluation and instantaneous evaluation to measure the impact of zoo visitors on seal behaviour. Both methods provided more or less similar results: under increasing visitor numbers, more seals spent their time submerged. We could also show that maximum daily temperature did not have a significant effect on the behaviour of seals in our study. While both methods yielded similar results for underwater swimming, social behaviour and resting on land; there were minor differences between the two methods for swimming with heads above the water and for scanning the environment. The first showed no effect of visitor numbers in the daily evaluation, but a significant decrease under increasing visitor numbers for the instantaneous evaluation. For scanning the environment the exact opposite was found, with a significant decrease in the daily evaluation and no significant effect of visitor numbers in the instantaneous evaluation. This may be a reflection of the random criterion to compare large crowds with small crowds. In the daily evaluation we only compared the seven days with highest visitor numbers with the seven days of lowest visitor numbers but we did not include the seven days in between, to measure only extremes (see also Kuhar 2008), but the cut off points were chosen randomly, and the effect size is in fact very small. On the other hand the discrepancies between daily and instantaneous evaluation do not seem to be caused by the "visitor effect / visitor attraction confound" since it is unlikely that increasing visitor numbers would be attracted by a less social interactions.

Ideally, physiological measures of stress should be included in analyses to evaluate animal welfare. Although we could not measure the direct impact of visitors on stress physiology of the seals, as in many studies on visitor effects (Davey 2007), our results did show that the seals were at least influenced by the number of visitors at their enclosure, both on an instantaneous and a daily scale. Including cortisol measures to evaluate stress in seals has happened in the wild (Constable *et al.* 2006) and this might be a fruitful approach to evaluate whether increasing visitor numbers cause increased stress, a method that has been applied to other zoo-housed mammals (Davis *et al.* 2005), but to our knowledge this has not yet happened in studies of captive seals.

It seems the seals in our study tried to avoid increasing visitors, by diving underwater, which in itself can be a means to reduce stress. Studies of visitor effects in other species have also reported reduced visibility under larger crowd conditions (Kuhar 2008). However, this finding is in contrast with other studies on the impact of visitors on seal behaviour, where increased visual scanning was observed under increased visitor numbers (Morgan and Tromborg 2007). Visual scanning can be considered as a positive visitor effect, or seals could perhaps be monitoring the environment, or begging for titbits. In our study, scanning gave somewhat inconclusive results: based on the daily evaluation we found that fewer seals scanned the environment under high visitor numbers.. In the instantaneous evaluation we did not find a significant influence of visitor numbers on the proportion seals that engaged in scanning behaviour.

We hypothesize that enclosure design is a crucial factor that influences scanning behaviour. In our study, scanning behaviour was relatively rare. The visitors were standing 1-1.5 m above the water level. It is possible that scanning occurs more when visitors are more at 'eye-level' of the seals. Similarly, swimming under water may be less useful for seals to evade visitors in exhibits with under-water viewing windows, which were not present in our study. Unfortunately no details on exhibit design are provided in the other studies of seals (Taylor *et al.* 1998; Morgan and Tromborg 2007). Interestingly, a study on captive Humboldt penguins also found more penguins submerged under higher visitor numbers (Condon *et al.* 2003). However, the authors consider this a positive visitor effect and suggest the penguins were attracted to the large and noisy crowds standing in front of an under-water viewing window. In the current study no such window was present, thus dispelling this explanation at least for the seals. We suggest that complex enclosures with visual barriers could be constructed to give seals a chance to move out of visitors' view if they choose, similar to what has been proposed for other species (Hosey 2000). Future research on visitor impact on seal behaviour, should focus experimentally providing and removing hideouts for seals, to assess the impact of such hideouts on seal behaviour. Ideally, measuring the hypothalamic-pituitary-adrenal axis activity to measure stress in captive seals should complement behavioural studies. In conclusion, the behaviour of seals in captivity seemed to be influenced by the number of visitors, both on a daily basis and an instantaneous level as seals hide underwater from increasing visitor numbers, but more information is needed to assess the effects of visitors on real welfare.

Acknowledgements

We thank the keepers and curator of marine mammals at Antwerp Zoo for assisting us in this research project. Dr. Linda Van Elsacker provided useful comments on the manuscript, and Evelien De Groot gave statistical advise. We thank the Flemish Government for structural and financial support of the CRC. We thank two anonymous reviewers for their comments on an earlier version of the manuscript.

References

Altmann, J. (1974) Observational study of behavior: sampling methods. *Behaviour* 49: 227-267.

Cassini, F.H. (2001) Behavioural responses of South American fur seals to approach by tourists: a brief report. *Applied Animal Behaviour* Science 71: 341-346.

Condon, E., Wehnelt, S. and Turner, Z. (2003) The effect of visitors on the behavior of Humboldt's penguins at Chester Zoo. *Federation Research Newsletter* 4(2): 3.

Constable, S., Parslow, A., Dutton, G., Rogers, T. and Hogg, C. (2006) Urinary cortisol sampling: A non-invasive technique for examining cortisol concentrations in the Weddell seal, *Leptonychotes weddellii*. *Zoo Biology* 25: 137-144.

Cook, S. and Hosey, G.R. (1995) Interactions sequences between chimpanzees and human visitors at the zoo. *Zoo Biology* 14: 431-440.

Davey, G. (2007) Visitors' effects on the welfare of animals in the zoo: A Review. *Journal of Applied Animal Welfare Science* 10: 169-183.

Davis, N., Schaffner, C.M. and Smith, T.E. (2005) Evidence that zoo visitors influence HPA activity in spider monkeys (*Ateles geoffroyii rufiventris*). *Applied Animal Behaviour Science* 90: 131-141.

Hosey, G.R. (2000) Zoo animals and their human audiences: what is the visitor effect? *Animal Welfare* 9: 343-359

Hosey, G.R. (2005) How does the zoo environment affect the behaviour of captive primates? *Applied Animal Behaviour Science* 90: 107-129.

Hosey, G.R. and Druck, P.L. (1987) The influence of zoo visitors on the behaviour of captive primates. *Applied Animal Behaviour Science* 18: 19-29.

Hunter, S.A., Bay, M.S., Martin, M.L. and Hatfield, J.S. (2002) Behavioral effects of environmental enrichment on harbor seals (*Phoca vitulina concolor*) and grey seals (*Halichoerus grypus*). *Zoo Biology* 21, 357-387.

Kovacs, K.M. and Inness, S. (1990) The impact of tourism on harp seals (*Phoca groenlandica*) in the Gulf of St Lawrence, Canada. *Applied Animal Behaviour Science* 26: 15-26.

Kuhar, C.W. (2008) Group differences in captive gorillas' reaction to large crowds. *Applied Animal Behaviour Science* 110: 377-385.

Margulis, S.W. and Westhus, E.J. (2008) Evaluation of different observational sampling regimes for use in zoological parks. *Applied Animal Behaviour Science* 110: 363-376.

Margulis, S.W., Soyos, C., and Anderson, M. (2003) Effect of felid activity on zoo visitor interest. *Zoo Biology* 22: 587-599:

Martin, P. and Bateson, P. (1993) *Measuring Behaviour: An introductory guide*. Cambridge: Cambridge University Press.

Mitchell, G., Tromborg, C.T., Kaufman, J., Bargabus, S., Simoni, R., Geissler, V. (1992) More on the 'influence' of zoo visitors on the behaviour of captive primates. *Applied Animal Behaviour Science* 35: 189-198.

Morgan, K.N. and Tromborg, C.T. (2007) Sources of stress in captivity. *Applied Animal Behaviour Science* 102: 262-302.

Ross, S.R., Lonsdorf, E.V., and Stoinski, T. (2007) Assessing the welfare implications of visitors in a zoo setting: A comment on Wells (2005). *Applied Animal Behaviour Science* 102: 130-133.

Taylor, A.A., Davis, H. and Boyle, G.J. (1998) Increased vigilance toward unfamiliar humans by harbor (*Phoca vitulina*) and gray (*Halichoerus grypus*) seals. *Marine Mammal Science* 14: 575-583.

Thompson, V. (1989) Behavioral response of 12 ungulate species in captivity to the presence of humans. *Zoo Biology* 8: 275–297.

van Polanen Petel, T., Giese, M., Hindell, M. (2008) A preliminary investigation of the effect of repeated pedestrian approaches to Wedell seals (*Leptonychotes wedellii*). *Applied Animal Behaviour* Science 112: 205-211.

Wells, D. (2005) A note on the influence of visitors on the behavior and welfare of zoo-housed gorillas. *Applied Animal Behaviour Science* 93: 13–17.

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Research Article

The Impact of housing and husbandry on the personality of cheetah (*Acinonyx jubatus*)

Kathy Baker[1,*] **and P. Kirsten Pullen**[2]

[1]*Newquay Zoo Environmental Park, Trenance Gardens, Newquay, Cornwall, TR7 2LZ*
[2]*BIAZA, Regent's Park, London, NW1 4RY, UK*
**Corresponding author: kathy.baker@newquayzoo.org.uk*

Keywords:
personality, cheetah, captive, keeper-animal relationships

Article history:
Received: 07August 2012
Accepted: 21 June 2013
Published online: 24 July 2013

Abstract

This study aimed to assess the effects of husbandry regimes on the personality of zoo-housed cheetahs (*Acinonyx jubatus*). 35 individual cheetahs from 7 zoos in the UK and Ireland were rated on 25 trait adjectives and 23 observable behaviours. Data on housing and husbandry variables were also collected. Behavioural observations during the addition of a novel object (a traffic cone) were also carried out. 11 of the personality and behaviour traits were determined as reliable using Intra Class Correlation coefficients (ICC[3, k]). Principal Components Analysis (PCA) using these traits resulted in the emergence of three personality dimensions, Dominance, Sociability and Keeper-directed Sociability. Animals' scores on the Dominance dimension were significantly negatively correlated with the frequency of touching the novel object and total time spent in contact with the novel object. Generalised linear mixed models were used to assess the effect of sex, age and husbandry variables on cheetahs' scores on each of the personality dimensions. Males scored significantly higher than females on the Dominance and Sociability dimensions. Age had no significant relationship with the personality dimensions. Three of the husbandry variables had significant relationships with personality dimensions. If the keeper entered the cheetah enclosure on a daily basis animals were scored significantly lower on the Dominance dimension. If prey was in sight animals scored higher on the Sociability and Keeper-directed Sociability dimensions. The percentage of barrier fence that the public had access to had a significant relationship with Keeper-directed Sociability, however the nature of this relationship was unclear potentially due to it being a confounded variable.

Data collected during this study resulted in the identification of three personality dimensions which are comparable with previous animal personality research. The personality dimensions showed some evidence of validity in correlations with behavioural measures; however the usefulness of novel object tests in validating personality assessments should be considered in future research. The study demonstrates that there may be important relationships between housing and husbandry variables and cheetah personality. Of particular importance may be the value of Keeper Animal Relationships (KARs) which have been identified in previous studies.

Introduction

The study of individual differences in non-human animals (here on referred to as animals) whether defined as personality, temperament, behavioural syndromes, or behavioural phenotypes, has received increased attention from researchers in recent years. Gosling's (2001) review identified a range of research documenting individual differences in behaviour that could constitute personality in species ranging from cephalopods to great apes. Although there are inherent difficulties in assessing personality dimensions within a range of species (Uher 2008), certain dimensions, such as Sociability have been identified repeatedly across species (Gosling 2001), and appear to readily translate within species across multiple situations (King *et al.* 2005; Weiss *et al.* 2007).

Historically research has focused on species held in laboratory (Capitanio 2011; Coleman 2011), and domestic (Anderson *et al.* 1999) environments and research topics have covered, amongst others, methodology (e.g. Stevenson-Hinde and Zunz 1978), health (see Cavigelli 2005 for a review), productivity (e.g. Muller and von Keyserlingk 2006) and welfare (e.g. Coleman 2011).

While the benefits of zoo based personality research for conservation have been identified (Bremner-Harrison *et al.* 2004; Watters and Meehan 2007; Powell and Gartner 2010), perhaps its most valuable use of personality assessments in the zoo setting is as a tool for animal husbandry and management. A survey of the personality of black rhinoceros (*Diceros bicornis*) in USA zoos suggests that personality can be affected by enclosure features, for example in males of the species there is a positive correlation with the percentage of public access (the percentage of the outdoor enclosure perimeter that allowed the public an unobstructed view of the enclosure) and the scores on a Fearfulness personality dimension. In addition personality may impact on breeding success as male rhinos' scores on Dominant and Olfactory personality dimensions were negatively related to breeding success, whilst females breeding success was negatively related to a personality dimensions labelled Chasing / Stereotypy / Mouthing. (Carlstead *et al.* 1999; Carlstead, Fraser *et al.* 1999).

One aspect of management that consistently reoccurs in Zoos is the introduction of new animals or the formation of new groups of animals. Kuhar *et al.* (2006) suggested that personality assessment of gorillas (*Gorilla gorilla*) may assist the

Species Survival Plan (SSP) breeding programme coordinators to make informed choices about the composition of new gorilla groups, especially all-male groups. They surveyed male gorillas in the AZA population, repeating the methodology used in an earlier study (Gold and Maple 1994) and found that individuals that were housed solitarily had significantly lower scores on an Understanding personality dimension than those in heterosexual groups. It is unclear, however, whether solitary individuals are rated lower on this dimension because they are solitary or whether they are housed solitarily because of some social component predictable by the Understanding factor. The authors suggest that if the Understanding factor is predictive of males that must be solitarily housed, the GBI would be a valuable tool for assessing social competence for establishing all-male gorilla groups.

Wielebnowski (1999) surveyed 44 adult captive born cheetahs in four North American breeding facilities and identified three major personality components (Tense-fearful, Vocal-excitable, and Aggressive). It was found that females scored higher on the Tense-fearful component than males, leading the authors to suggest that a high level of fearfulness maybe an important adaptive trait, particularly in an open habitat and where lion density is high. An analysis of the breeding success of the sample population revealed that non-breeders of both sexes scored significantly higher on the Tense-Fearful component than breeders. Wielebnowski suggests that these results may therefore also allow us to predict an individual's ability to reproduce in a captive environment; implying that animals that score more highly on the Tense-fearful may not be as adaptive to a captive environment, and may need more secluded enclosures and provision of hiding places in order to breed successfully.

In captive small felids there is a significant positive relationship between reproductive success and the time keepers spend interacting with their animals (Mellen 1991). These results suggest that animals learn to be comfortable with the inevitable presence of their human caretakers, and may therefore score less highly on the tense-fearful dimension. In effect a positive Keeper Animal Relationship (KAR) can benefit both the animal and the keeper. This has not been extensively researched in zoo animals (although see Claxton 2011), despite a growing amount of literature in domesticated animals (Carlstead 2009).

While there is a growing body of personality research occurring within the zoo world (Carlstead *et al.* 1999a, 1999b; Powell and Svoke 2008; Weiss *et al.* 2007), there is a feeling that greater knowledge of an individual animal's personality will allow us to not only to assess an animal's needs, but also to tailor situations or roles in captivity to suit an individual animal's personality (Watters and Powell 2011). In order to achieve this we need to have a greater understanding of the relationship between an animal's environment and its personality.

The aims of this study were to *i)* survey the personalities of the UK population of cheetah to see if we can identify similar behavioural components to the Wielebnowski study and *ii)* to evaluate whether there are any relationships between housing and husbandry factors and *A. jubatus* personality.

Materials and Methods

Study Design

Questionnaires were sent to 11 zoos in the UK and Ireland holding cheetah as recorded by ISIS (International Species Information System) at the end of 2002. Seven institutions returned questionnaires, a response rate of 64%, providing data on a total of 34 individual cheetahs (16 males and 18 females). Behavioural observations during novel object tests for validation purposes were conducted at 6 institutions on 29 individuals (15 males and 14 females).

Questionnaires

A questionnaire was constructed in order to evaluate cheetah personality. The questionnaire had three sections. Section 1, consisted of 25 trait adjectives with accompanying behavioural definitions, e.g. 'Cautious - Approaches novel objects/ situations with care and wariness'. Section 2, consisted of 23 observable behaviours e.g. faecal marking or sharpening claws. Raters had to rate each adjective/behaviour on a scale from 1 (does not apply to the cheetah in question) to 7 (applies strongly to the cheetah in question). The third section gathered information of the environment of the cheetahs, including details on housing and husbandry. The questionnaires were accompanied by detailed instructions for the raters to ensure they were filled out as accurately as possible.

Novel Object Tests

At the six zoos used for behavioural data collection, a novel object test was used to validate the questionnaire data; a small traffic cone was used as a novel object. After the object had been placed in the enclosure instantaneous scan sampling at one-minute intervals was carried out for 30 minutes. Latency to approach the object was recorded as was the frequency of contacts, and the total time spent in contact with the object. Due to the nature of housing and husbandry routines some individuals were tested alone while others were tested in social groups although data was collected for all individuals.

Data analysis

Inter-observer reliability

To determine the within-subject reliability the Intra Class Correlation (ICC [3,k]) scores were calculated using each individual cheetah's ratings (Shrout and Fleiss 1979). An ICC [3,k] of > 0.60 indicates good agreement between observers (Shrout and Fleiss 1979) therefore any animals that had and ICC [3,k] below this value were discarded from further analysis. To determine across-subject reliability the data for 32 animals at six zoos that were rated by more than one observer were used to calculate ICC coefficients for each trait. An average ICC value for each trait was then calculated and any trait that had a mean value below 0.60 was removed from further analysis.

Principal Components Analysis

All the reliable traits were entered into an Exploratory Principal Components Analysis (PCA) using the statistical computer programme SPSS vs. 17. Oblique (direct Oblimin) rotation was used as this is an exploratory analysis and there is reason to believe that factors may be correlated. The Eigenvalues above one, interpretation of the scree plot and parallel analysis were used to establish the number of components that should be retained (O'Connor 2002). The pattern matrix output was used for interpretation of component loadings.

Validity analysis

A score for each animal on each of the personality dimensions from the PCA analysis was calculated by averaging their scores on each of the individual traits that had salient (>0.40) loadings on a particular dimension (for negatively loaded traits a reverse score was calculated by taking the original score from 8). If a trait had salient loadings on more than one dimension then it was assigned to the dimension on which it had the higher loading. Pearson product-moment correlations were then used to examine the relationship between personality dimensions and the behaviour measures recorded during the novel object test.

Relationship between personality and environmental variables

The effects of housing and husbandry on the personality factors were analysed with Generalised Linear Mixed Models using SPSS 19 statistical software (SPSS 2010, IBM, Chicago, IL, U.S.A). All factors were run on a normal distribution (residuals were tested for normality and no significant differences were found). Fixed factors included age, gender, percentage of the enclosure barrier that is solid (solid barrier), percentage of the barrier that the public have access to (public perimeter), whether a prey species was in sight of the cheetahs and whether the keepers enter the enclosure with the animals daily (keeper enters daily).

The model was fitted using a backwards stepwise process to find the minimum adequate model for explaining the variance observed in the response variable (Bolker *et al.* 2009). Only significant explanatory variables were retained and the models with the lowest Akaike's Information Criterion were interpreted. All tests are two-tailed. For appropriate significant results post-hoc pairwise comparisons with Bonferroni corrections were carried out.

Results

Inter-observer reliability

ICC values for each individual animal ranged from 0.63 to 0.98 (Table 1). No animal had an ICC value below 0.60 therefore all animals' data was used in further analysis. ICC values for each trait that met the mean >0.60 threshold are presented in Table 2. 11 traits were found to reach the criterion. While the reliability analysis could only be done using the data from six zoos (32 animals) due to the sampling constraints, if the traits were found to be reliable they were assumed to be reliable across all seven zoos, so that all the personality data could be entered into the PCA analysis.

Zoo	Number of raters	Sex	Age (years)	ICC [3,k]
Fota Wildlife Park, Ireland	2	Female	11.6	.94
		Female	9.8	.92
		Female	2.4	.81
		Female	5.8	.93
		Male	9.8	.98
		Male	2.4	.90
		Male	1.1	.93
		Male	5.8	.94
		Male	4.0	.94
		Male	5.8	.98
Marwell Wildlife, UK	2	Male	12.9	.82
		Female	3.8	.72
		Female	10.9	.86
Paignton Zoo Environmental Park, UK	2	Male	4.3	.83
		Male	4.3	.93
		Female	3.8	.89
		Female	3.8	.65
Colchester Zoo, UK	2	Female	7.8	.95
		Female	1.8	.92
		Male	1.8	.92
		Male	1.8	.94
		Male	1.8	.92
Belfast Zoological Gardens, UK	1	Male	4.9	-
		Female	1.5	-
		Female	1.5	-
Hamerton Zoo Park, UK	2	Male	3.5	.82
		Female	4.8	.63
		Male	6.2	.65
		Female	6.8	.68
		Male	1.7	.68
		Female	1.7	.79
Whipsnade Zoo, UK	2	Female	11.0	.96
		Female	13.3	.97
		Female	10.6	.94

Table 1. Summary of study animals and within subject reliability ICC [3,k] .

Principal Components Analysis

The 11 reliable traits were entered into an exploratory Principal Components Analysis (PCA). Based on the component retention criteria three factors were retained which accounted for 63.12% of the variance (Table 3). Based on the individual items that loaded onto each component (from now on referred to as personality dimensions) they were labelled, Dominance, Sociability and Keeper-directed Sociability (traits loading on this last dimension refer only to the cheetah's interactions with keepers).

Trait	Institution*						
	I	II	III	IV	V	VI	Mean
Friendly to you	.95	.82	.99	.94	.99	.67	.90
Aggressive to you	.84	.86	.85	.00	.93	.75	.71
Assertive	.93	.89	.76	.30	.93	.89	.78
Dominant to conspecifics	.98	.94	.38	.89	.84	.97	.83
Playful	.98	.86	.86	.59	.84	.86	.83
Timid/shy	.98	.63	.00	.40	.80	.99	.63
Head rubbing	.93	.86	.87	.59	.49	.99	.79
Close follow	.98	.86	.57	.00	.75	.99	.69
Contact aggression	.95	.86	.57	.00	.76	.92	.68
Chirp-fear	.78	.67	-.25	.87	.80	.77	.61
Stutter	.85	.99	.73	.36	.00	.98	.65

**Institutions: I=Fota; II=Marwell; III=Colchester; IV=Paignton; V=Hamerton; VI=Whipsnade*

Table 2. Intra-Class Correlation Coefficients ICC[3,k] for all traits with a mean ICC >0.60.

Trait	Dominance	Sociability	Keeper-directed Sociability
% Variance explained	30.80	18.03	14.32
Contact aggression	**.89**	.16	-.08
Assertive	**.74**	**.44**	.07
Dominant to conspecifics	**.69**	.23	.06
Chirp-fear	**.66**	-.12	-.02
Stutter	**.63**	.01	.01
Head rub	.03	**.86**	.05
Timid/shy	-.13	**-.79**	.22
Playful	**.60**	**.60**	.04
Close follow	.16	**.53**	.15
Stutter bark	**.41**	**.51**	-.19
Aggressive to you	.11	.11	**-.91**
Friendly to you	.02	.01	**.90**

Table 3. Results of the principle components analysis. Salient loadings >0.4 are in bold.

Validity Analysis

Dominance personality scores were negatively correlated with the frequency of contacts ($r = -0.537$, $n = 29$, $p < 0.01$) and total time in contact with the novel object ($r = -0.64$, $n = 29$, $p < 0.01$). Sociability and Keeper-directed Sociability scores were not significantly correlated with any of the behavioural measures.

Sex, Age, Housing and Husbandry Analysis

Dominance

There was a significant effect of gender on Dominance score (Wald's $\chi^2 = 5.41$, df = 1, $p = 0.02$), with males scoring more highly than females (means: males= 3.59, females = 2.88). There was a significant effect of whether the keepers enter daily (Wald's $\chi^2 = 12.35$, df = 1, $p < 0.01$), with individuals where the keeper does not enter daily scoring more highly than individuals where the keeper does enter daily (means: keeper does not enter daily = 3.82, keeper enters daily = 2.64). There was also a significant effect of whether prey was in sight (Wald's $\chi^2 = 18.99$, df = 1, $p < 0.01$), with individuals where prey is in sight scoring more highly than individuals where prey is not in sight (means: prey in sight = 4.02, prey not in sight = 2.44).

Sociability

There was a significant effect of gender on sociability (Wald's $\chi^2 = 7.89$, df = 1, $p = 0.01$), with males scoring more highly than females (means: males = 3.99, females = 3.03).

Keeper-directed Sociability

There was a significant effect of whether prey was in sight on keeper-directed sociability (Wald's $\chi^2 = 10.47$, df = 1, $p = 0.01$), with individuals where prey is in sight scoring more highly than those where prey is not in sight (means: prey in sight = 3.55, prey not in sight = 1.93). There was also a significant effect of the amount of perimeter that the public had access to (Wald's $\chi^2 = 47.14$, df = 6, $p < 0.01$). However pairwise post-hoc tests did not reveal any easily describable relationships between percentage of perimeter and keeper-directed Sociability.

Discussion

The results suggest that keepers can reliably assess individual cheetahs on a range of personality and behaviour traits. Using these ratings three personality dimensions were identified within the surveyed population of cheetah, Dominance, Sociability and Keeper-directed Sociability. One of these dimensions, Dominance, demonstrated good construct validity as individual dimension scores had significant correlations with behavioural measures recorded during novel object tests.

Structure of personality dimensions

Dominance was the strongest personality dimension to emerge from the PCA analysis accounting for 31 % of the variance in the data. Animals rating highly on this dimension scored higher on the individual behavioural traits contact aggression, assertive, dominant to con-specifics, chirp-fear and stutter. Analogous personality dimensions have been found in a wide range of animals including for example the Dominance dimension in chimpanzees (*Pan troglodytes*) (King and Figueredo 1997), and the Assertiveness dimension in hyenas (*Crocuta crocuta*) (Gosling 1998). The Dominance dimension in the current study is not directly comparable with any one dimension in the Wielebnowski (1999) study however it most closely resembles the Aggressive dimension as it contains traits relating to aggressiveness. One of the potential reasons for not finding dimensions directly comparable with the Wielebnowski study is the trait list that was used. The trait list in the current study was generated using an eclectic approach (Uher 2008), this involved first using the Wielebnowski trait list as a starting point for trait generation but traits were added based on other animal personality research and the behavioural repertoire of cheetah. Therefore a greater range of traits was used; 48 traits in the current study compared to 18 in the Wielebnowski study. This finding stresses the importance of the trait lists that are used for personality studies, a topic which has been highlighted in primate research. When a trait list designed for rhesus macaques (*Macaca mulatta*) (Stevenson-Hinde and Zunz 1978) trait list was used to assess personality in *P. troglodytes* three personality dimensions emerged (Murray 1998), however in the same species when using a trait list derived from that of the human Five Factor Model (FFM), six personality dimensions emerged (King and Figueredo 1997). Uher and Asendorpf (2008) suggest that empirical differences were not caused by species personality differences but by the personality dimensions that were covered by the list of trait adjectives. The current study demonstrates that trait list generation is also an important factor when interpreting results from felid personality research.

Animals rating highly on Sociability scored higher on the individual behavioural traits head rub, playful, close follow, stutter bark, and lower on the trait timid/shy. Of the three dimensions identified in the Wielebnowski (1999) study the Sociability dimension in the current study most closely resembles the Vocal-excitable dimension which has high positive loadings on variables such as playful and active. Sociability is one of the most commonly assessed personality dimensions in animal personality research and has been found across a wide range of taxa, including felid species (Freeman and Gosling 2010; Gosling 2001). Therefore we would expect to find a sociability type dimension in cheetah.

Animals rating highly on Keeper-directed Sociability scored higher on the individual trait friendly to you and lower on the individual trait aggressive to you. There are very few published studies that have included keeper-directed traits in animal personality research however a human-directed Agreeableness factor has been found in spotted hyenas (*C. crocuta*) (Gosling 1998); and a Friendly to keeper dimension was found in zoo housed black rhino (*D. bicornis*) (Carlstead *et al.* 1999a). In the Wielebnowski (1999) study the trait Aggressive to people trait loaded highly on an Aggressive personality dimension in zoo-housed cheetahs (*A. jubatus*) (Wielebnowski 1999). Gosling (1998) suggests that, in the case of hyenas (*C. crocuta*), the presence of a human-directed Agreeableness dimension may be "manifestations of individual differences in the more general domain of social sensitivity" (p. 113), as hyena exhibit complex social relationships. We may therefore expect to find similar dimensions in other carnivore species that exhibit any kind of social relationships. In addition this dimension has a plausible connection with the Keeper Animal Relationship (KAR) (Claxton 2011) which can be extremely important in the management of captive carnivores (Mellen 1991). The current study did not include any analyses of KARs but this may be an important area of future research.

Validity of identified personality dimensions

Of the three personality dimensions revealed in this study Dominance was the only one to show significant correlations with any of the behavioural measures recorded during novel object tests. Animals scoring highly on the Dominance dimension had fewer bouts of contact with the novel object and spent less overall time in contact with the object than animals scoring lower on this dimension. We might expect that animals rating highly on this dimension would spend more time inspecting novel objects due to their high rating on the individual trait assertive, i.e. they may be more inclined than their low Dominance counterparts to investigate novel, potentially threatening, stimuli. The choice of novel object in the current study may be an important factor when interpreting this result further, a traffic cone, while novel, may not be particularly threatening therefore Dominant animals may be more inclined to ignore this object when it is presented to them. Carter *et al.* (2012) assessed Boldness in wild chacma baboons (*Papio ursinas*) using response to two different objects; a model puff adder (*Bitis arietans arietans*), and a novel food item. They found that animals' behavioural responses to the two different objects did not correlate across the two contexts and that responses were potentially indicative of anxiety rather than Boldness. This study highlights the importance of choosing novel objects which are relevant to the particular dimension of interest, potentially future studies should first explore the personality dimensions and then design behavioural tests for validation purposes.

The effect of housing and husbandry on personality traits

Housing and husbandry routines had a significant impact on two of the identified personality dimensions, Dominance and Keeper-directed Sociability. Dominance scores were significantly lower if keepers entered the enclosure with the cheetahs on a daily basis. In comparison Wielebnowski (1999) found that it was the Tense/Fearful dimension that was significantly affected by the keepers entering the enclosure, with animals being less Tense/Fearful when entering the enclosure with the animal was part of the day to day husbandry routine. Although a Tense/Fearful dimension was not identified in this research, it is reasonable to assume that cheetahs housed where the keepers enter the enclosure score less on the Dominance dimension because they are less fearful of the keepers. Freeman and Gosling's (2010) review of studies in primate personality found that Dominance related traits were classified under Fearfulness, Dominance or Independence dimensions by various authors, and that Fearfulness and Dominance frequently overlapped. Within this research, aggressive behaviours loaded on the Dominance dimension but it has been well documented in several species that fearful animals may respond in an aggressive manner (e.g. dogs, King *et al.* 2003). Animals (particularly small felids) which develop a positive relationship with their keepers and show a less fearful response will often demonstrate improved responses to environmental change in the enclosure and increased breeding success (Mellen 1991).

It should be noted that keeper perceptions of animals may be an important factor when interpreting the above results. It is reasonable to assume that whether keepers enter the enclosure daily is dictated by traditional husbandry routines at individual zoos rather than because keepers believe that individual cheetahs are more or less Dominant. Certainly there were no replies to the questionnaire suggesting that keepers would choose to enter with some cheetahs over others based on the Dominance behaviour of the individual animal. This may mean that because the keepers enter the enclosure daily they perceive their animals in a different way to keepers that do not carry out this management practice. In the current study we do not believe that keeper perceptions of animals is affecting the results as if this was the case we would expect to see similar relationships with other personality dimensions, especially the Keeper-directed Sociability dimension.

Dominance and Keeper-directed Sociability scores were both also significantly affected by the housing of a prey species in sight of the cheetahs. Individuals that could see prey species scored more highly on both these dimensions. There is very little published data on the behavioural impact of housing a prey species in view of a predator and there is no immediately obvious reason why these relationships should occur. In the case of Dominance potentially there may be increased levels of frustration through not being able to perform a natural behaviour for which there is a high motivation (Dawkins 1988) i.e. viewing a prey species but not being able to hunt, and it may be reasonable to suggest that higher levels of Dominance behaviours may result from the frustration of not being able to get to a potential prey item. In the case of Keeper-directed Sociability cheetahs may associate keepers with food so watching a prey species may promote excitability towards the keepers. Potentially it could be a misinterpretation of behaviour on the keepers' part. The cheetah may be generally more excitable when housed within sight of the prey species, the keepers may only be aware of this from observing their behaviour as they approach the enclosure. Certainly it has been documented that housing felid species in view of potential prey species can increase levels of activity, more often stalking behaviour (Lyons *et al.* 1997).

Although there is an effect of the percentage of the perimeter fence on the level of Keeper-directed Sociability, there is not a clear relationship. Percentage of the perimeter fence that public have access to is potentially a confounded variable as it shows a correlation with other variables such as enclosure size. It is therefore not possible from these data to explore this relationship further.

The effect of age and gender

There was no significant effect of age on any personality dimensions. This contrasts to previous research with other felid species (e.g. snow leopards, *Uncia uncia*, Gartner and Powell 2011), however does agree with results from the U.S. cheetah population (Wielebnowski 1999). This non-significance in the current research may be due to the small sample size so further study would need to be done to confirm this finding.

There were significant effects of gender on the Dominance and Sociability dimensions. In both cases males score more highly on the dimensions than the females. This indicates that males are both more dominant and more sociable than females. Male cheetahs are known to only associate with females during oestrous where they will monopolise them to prevent multiple matings (Gotelli *et al.* 2007). Additionally cheetahs are known to be unusual amongst big cats due to the existence of permanent male coalitions coupled with the defence of small portions of a female's home range (Gotelli *et al.* 2007). It is therefore reasonable to assume that male cheetahs would score more highly on both the Dominance and Sociability dimensions than females.

However it must be noted that within our relatively small sample size different zoos implement different social housing conditions, for example during the time of data collection Fota Wildlife Park held ten animals, six of which were male and housed together, four of which were female and were rotated individually on exhibit for breeding purposes. Inherently this means that the males within our test sample would have been housed in a situation that would encourage sociability to a greater extent than the housing of the females.

Implications for management

One of the main issues facing cheetah management in captivity is the lack of breeding success. While we have not looked specifically at this due to the small sample size of successful breeding groups within the data set, our results could be used as a starting point for future management recommendations. For example our results suggest that building a relationship with your animals can reduce Dominance personality scores and therefore associated behaviours, but housing them in sight of prey can increase Dominance personality scores. However further research on the effect of Dominance personality scores on breeding success is needed before firm recommendations can be made.

One other important recommendation from the current research is the development of a trait list that is tested for reliability and validity across a range of felid species would be beneficial. This could consist of a short list of core personality traits (that are established to be reliable and valid), so cross-study and cross-species comparisons can be made, and an additional list of species-specific traits if they are needed by the researcher.

Acknowledgements

The authors would like to acknowledge the managers and keepers of the 7 UK and Irish zoos that participated in this research; Fota Wildlife Park, Marwell Wildlife, Hamerton Zoo Park, Paignton Zoo Environmental Park, Colchester Zoo, Belfast Zoological Gardens, Whipsnade Zoo. The authors would also like to knowledge the assistance of Sheila McKay (Manchester University) for implementing and collating the surveys. This research was undertaken with grants from the Universities Federation for Animal Welfare (UFAW) and the Association of British Wild Animals Keepers (ABWAK).

References

Anderson, M. K., Friend, T. H., Evans, J. W. and Bushong, D.M. (1999) Behavioral assessment of horses in therapeutic riding programs. *Applied Animal Behaviour Science* 63(1): 11-24.

Bremner-Harrison, S., Prodohl, P. A. and Elwood, R. W. (2004). Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). *Animal Conservation* 7(3): 313-320.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. and White, J. S. S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* 24(3): 127-135.

Capitanio, J. P. (2011) Individual differences in Emotionality: Social temperament and health. *American Journal of Primatology* 73: 507-515

Carlstead, K., Mellen, J. and Kleiman, D. G. (1999) Black rhinoceros (*Diceros bicornis*) in U.S. zoos I: Individual behaviour profiles and their relationship to breeding success. *Zoo Biology* 18: 17-34.

Carlstead, K., Fraser, J., Bennett, C. and Kleiman, D. G. (1999) Black rhinoceros (*Diceros bicornis*) in U. S. zoos II: Behavior, breeding success and mortality in relation to housing facilities. *Zoo Biology* 18: 35-52

Carlstead, K. (2009) A comparative approach to the study of keeper-animal relationships in the zoo. *Zoo Biology* 28(6):589- 608

Carter, A. J., Marshall, H. H., Heinsohn, R. and Cowlishaw, G. (2012) How not to measure boldness: novel object and antipredator response are not the same in wild baboons. *Animal Behaviour* 84(3): 603-609.

Cavigelli, S. A. (2005). Animal personality and health. *Behaviour* 142: 1223-1244.

Claxton, A. M. (2011) The potential of the human-animal relationship as an environmental enrichment for the welfare of zoo-housed animals. *Applied Animal Behaviour Science* 133: 1-10.

Coleman, K. (2011) Individual differences in temperament and behavioural management practices for nonhuman primates. *Applied Animal Behaviour Science* 137(3-4): 106-113.

Dawkins, M. S. (1988) Behavioural deprivation: A central problem in animal welfare. *Applied Animal Behaviour Science* 20(3-4): 209-225.

Freeman, H. D. and Gosling, S. D. (2010) Personality in nonhuman primates: A review and evaluation of past research. *American Journal of Primatology* 72: 653-671.

Gartner, M. C. and Powell, D. (2011) Personality assessments in snow leopards (*Uncia uncia*) *Zoo Biology* 31(2): 151-165.

Gold, K. C. and Maple, T. L. (1994) Personality assessment in the gorilla and its utility as a management tool. *Zoo Biology* 13(5): 509-522.

Gosling, S. D. (1998) Personality dimensions in spotted hyenas (*Crocuta crocutas*). *Journal of Comparative Psychology* 112(2): 107-118.

Gosling, S. D. (2001) From mice to men: What can we learn about personality from animal research. *Psychological Bulletin* 127(1): 45-86.

Gotelli, D., Wang, J., Bashir, S. and Durant, S. M. (2007) Genetic analysis reveals promiscuity among female cheetahs. *Proceedings of the Royal Society* B 274: 1993-2001.

King, J. E. and Figueredo, A, J. (1997) The five-factor model plus dominance in chimpanzee personality. *Journal of Research in Personality* 31:257-271

King, J. E. and Landau, V. I. (2003) Can chimpanzee (*Pan troglodytes*) happiness be estimated by human raters? *Journal of Research in Personality* 37: 1-15.

King, J. E., Weiss, A. and Farmer, K. H. (2005) A chimpanzee (*Pan troglodytes*) analogue of cross-national generalization of personality structure: zoological parks and an African sanctuary. *Journal of Personality* 73: 389-410.

Kuhar, C. W., Stoinski, T. S., Lukas, K. E. and Maple T. (2006) Gorilla behaviour index revisited: Age, housing and behaviour. *Applied Animal Behaviour Science* 96: 315-326.

Lyons, J., Young, R. J. and Deag, J. M. (1997) The effects of physical characteristics of the environment and feeding regime on the behavior of captive felids. *Zoo Biology* 16(1): 71-83.

Mellen, J. D. (1991) Factors influencing reproductive success in small captive exotic felids (*Felis* spp.): A multiple regression analysis. *Zoo Biology* 10(2): 95-110.

Müller, R. and von Keyserlingk, M. A. G. (2006) Consistency of flight speed and its correlation to productivity and to personality in *Bos Taurus* beef cattle. *Applied Animal Behaviour Science* 99(3-4): 193-204.

Powell, D. M. and Svoke, J. T. (2008) Novel environmental enrichment may provide a tool for rapid assessment of animal personality: A case study with giant pandas (*Ailuropoda melanoleuca*). *Journal of Applied Animal Welfare Science* 11(4): 301-318.

Powell, D. M. and Gartner, M. C. (2011). Applications of personality to the management and consevration of nonhuman animals. In: Inoue-Murayama, M. Kawamura, S. and Weiss, A. (Eds) *Primatology Monographs: From genes to animal behaviour: Social Structure, Personalitues, Communication by Colour*, Springer, Tokyo (2011). pp. 185-199.

Shrout, P. E. and Fleiss, J. L. (1979) Intraclass correlations: Uses in assessing rater reliability. *Psychological Bulletin* 86(2): 420-428.

Stevenson-Hinde, J., & Zunz, M. (1978).Subjective assessment of individual rhesus monkeys. *Primates* 19: 473-482.

Uher, J. (2008) Comparative personality research: Methodological approaches. *European Journal of Personality* 22: 427-474.

Watters, J. V., and Meehan, C. L. (2007) Different strokes: Can managing behavioural types increase post-release success? *Applied Animal Behaviour Science* 102(3-4): 364-379.

Weiss, A., King, J.E. and Hopkins, W.D. (2007) A cross-setting study of chimpanzee (*Pan troglodytes*) personality structure and development. *American Journal of Primatology* 69(11): 1264-1277.

Wielebnowski, N. J. (1999) Behavioural difference as predictors of breeding status in captive cheetahs. *Zoo Biology* 18: 335-349.

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Evidence based practice

A therapeutic environmental enrichment programme for managing pathological behaviour in the fossa (*Cryptoprocta ferox*)

Raffaele Grisa[1], **Marina Bertelé**[1], **Stefano Vaglio**[1,2,*] **and Caterina Spiezio**[1]

[1] Research and Conservation Department, Parco Natura Viva - Garda Zoological Park; Località Figara 40, 37012 Bussolengo (VR), Italy.
[2]Laboratori di Antropologia, Dipartimento di Biologia; Università degli Studi di Firenze; Via del Proconsolo 12, 50122 Florence, Italy.
**Corresponding author: stefano.vaglio@parconaturaviva.it*

Keywords:
Fossa, enrichment, behaviour management

Article history:
Received: 30 May 2012
Accepted: 01 October 2012
Published online: 24 July 2013

Abstract

This study is based on an adult male fossa (*Cryptoprocta ferox*) that arrived at Garda Zoological Park (Italy) exhibiting stereotyped and self-injuring behaviours and follows its subsequent rehabilitation through a long-term environmental enrichment programme. Data were collected over a period of six months. Continuous focal animal sampling was used to collect behavioural data during 90-minute sessions; 24 sessions took place over the first two months and two sessions took place six months after his arrival. Data were analysed using non-parametric tests. At the beginning of the study period, the predatory behaviour of the fossa was not species-specific since he was not able to find food items when they were hidden in the enclosure. It usually interacted with items for just a few minutes. Rubbing different scents on enclosure furniture promoted play only for a few minutes. After two months of behavioural observations, its behaviour only improved slightly and stereotyped and self-injuring behaviours were maintained. However, we continued the intense enrichment programme for a longer period. Six months after its arrival rare undesirable behaviours or noticeable side effects were observed whilst species-specific behaviours were recorded. Results showed that the environmental enrichment curtailed the aberrant behaviours. In conclusion, this case study provides evidence supporting the hypothesis that a suitable enrichment programme could have a therapeutic effect on pathological behaviour in captive animals.

Background

Studies on the effects of environmental enrichment are largely limited to zoo captive animals. Their findings confirm evidence deriving from researches on laboratory animals by showing that enrichment programmes are able to promote both brain plasticity and animal welfare (Stewart and Bayne 2004). In particular, zoo environmental enrichment can be used to improve the physical and psychological well-being of captive animals (Young 2003).

The assessment of an animal's psychological well-being is based on the response of the animal to its environment and on the animal's ability to cope effectively with day-to-day changes in its social and physical environment. Furthermore, the assessment must be based on the absence of maladaptive or pathological behaviour that results in self-injury or other undesirable consequences and on the ability to engage in beneficial species-typical activities (Mason *et al.* 2007). In captivity, zoo animals have been known to exhibit stereotyped behaviours, which are characterised as behavioural patterns that are repetitive, invariant, and with no obvious function (Mason 1991). These patterns are thought to be caused ultimately by artificial environments that do not allow animals to satisfy their typical behavioural needs and can actually cause harm to the animals (Clubb *et al.* 2006). Enrichment is one of the most common approaches to enhance the quality of captive animal care and, therefore, to improve the animal's physical and psychological welfare (Shepherdson *et al.* 1998).

In March 2009, a captive adult male fossa (*Cryptoprocta ferox*) arrived at Parco Natura Viva - Garda Zoological Park (Italy). In the wild the fossa is a solitary species active both during the day and at night. Its diet consists mostly of small mammals and birds, but also includes reptiles, frogs and insects. It is a powerful predator, and has keen senses of vision, hearing and smell (i.e. variable home range which they scent mark with secretions from their anal glands). Moreover, it is an excellent climber, and uses its long tail to provide balance when pursuing prey through the trees (MacDonald 2001). On arrival, his behaviour was not species-specific. Indeed, even if the enclosure was supplied with climbing apparatus, the fossa never exhibited climbing ability using branches and relatively fine ropes. Furthermore, he did not seem able to properly climb trees and leap from branch to branch, as his body did not appear heavily muscled and his tail did not act as a balancing organ. In addition, this individual showed serious behavioural problems such as agitation, high levels of repetitive pacing, and pathological behaviours that resulted in self-injury and self-mutilation. The behaviours of this individual resembled those of humans with post-traumatic stress disorder (e.g. Mallonée and Joslin 2004).

Action

The space available to the fossa was structured, usable, and complex and there was also structural enrichment. In addition, a very intensive enrichment programme was planned to improve the physical and psychological well-being of this animal. A variety of whole food items was presented to encourage usual food processing behaviours by the fossa. Chicken, rabbit, quail, and eggs were hidden in the enclosure. Manipulative items (i.e. logs, plastic barrels, cardboard boxes, etc.) were provided and different scents (i.e. spices, herbs, etc.) were rubbed on the enclosure furniture. This type of enrichment programme was planned to enhance species-specific behaviours, such as exploration and olfactory behaviours, by providing different kinds of stimuli. In addition, the programme involved a positive reinforcement training (PRT) session, on the basis of positive rewards (i.e. pieces of meat), to train the fossa to discontinue the repetitive pacing behaviour by performing species-specific behaviours. Participating in appropriately designed training sessions can give animals some amount of control in their lives.

The 60-min PRT session was carried out once a day, in the morning (from 8:30 am to 9:30 am). Non-feeding (manipulative objects, different scents, exhibit changes, etc.) and feeding enrichments were alternately provided once a day in the mid-morning (11:00 am). The 90-min data collection started immediately after the enrichment session (from 11:00 am to 12:30 am).

Data collection started immediately after arrival of the fossa (March 19, 2009) and lasted over a period of six months. Since the fossa behaviour was not species-specific, a behavioural report was transmitted to the EEP coordination and the very intensive enrichment programme was planned. Statistical analysis involved those data collected from April 13 to September 9, 2009, parallel to the implementation of the programme. Over this period behavioural data were collected through 90-minute focal animal sessions (26 sessions in total). Twenty-four sessions took place over the first two months and two sessions took place six months after his arrival. Behaviours showed in the first (12 sessions) and the second month (12 sessions) and, on the other side, in the "first phase" (first two days of the first two months), "last phase" (last two days of the first two months), and "post phase" (six months after his arrival), were compared to analyse the behavioural progresses of the fossa over the period. Time duration of behaviours was sampled. Data were analysed using non-parametric tests and the single-case approach.

The single-case approach refers to researches in which the phenomena of interest are studied using a single subject (also referred to as N-of-1 designs or single-subject research). Single-case research designs have a long history in psychological science, which demonstrates the enduring value of such approaches (Barlow and Hersen, 1984). In single-case designs, data derive from a single case or small group of cases and there are multiple observations per individual per condition, so that the commonly used group design methods are not appropriate.

Non-parametric tests such as randomization tests are useful in single case studies and the magnitude (e.g. mean) of behavioural performance for each condition can be appropriately evaluated (Box *et al.* 1994). Over the last few years the statistical approach to single-case data analysis has increased in popularity and sophistication (Franklin *et al.* 1997).

In all tests, the significance level was set to 0.05 (Siegel and Castellan 1992). StatView for Windows and Macintosh (version 5.0) was used for all the analyses.

Consequences

Fossa behaviours over a two month period

Results suggested that stereotyped were the most common behaviours. There was a decrease in stereotyped behaviours between the first month (12 sessions) and the second month (12 sessions) of behavioural observations. Furthermore, there was a significant increase in enrichment interaction (Wilcoxon Test: Z = -1.988; P = 0.0469) and foraging (Wilcoxon Test: Z = -2.803; P = 0.0051) and a significant decrease in locomotion (Wilcoxon Test: Z = -2.275; P = 0.0229) in the two month period. Nevertheless, the stereotyped (STE) behaviour was always more common than other behaviours such as scent-marking (MAR), exploration (EXP), inactivity (INA), locomotion (LOC), and foraging (FOR) (Figure 1).These results underline some beneficial effects on fossa behaviour, provided by the enrichment programme, even if the changes were very subtle.

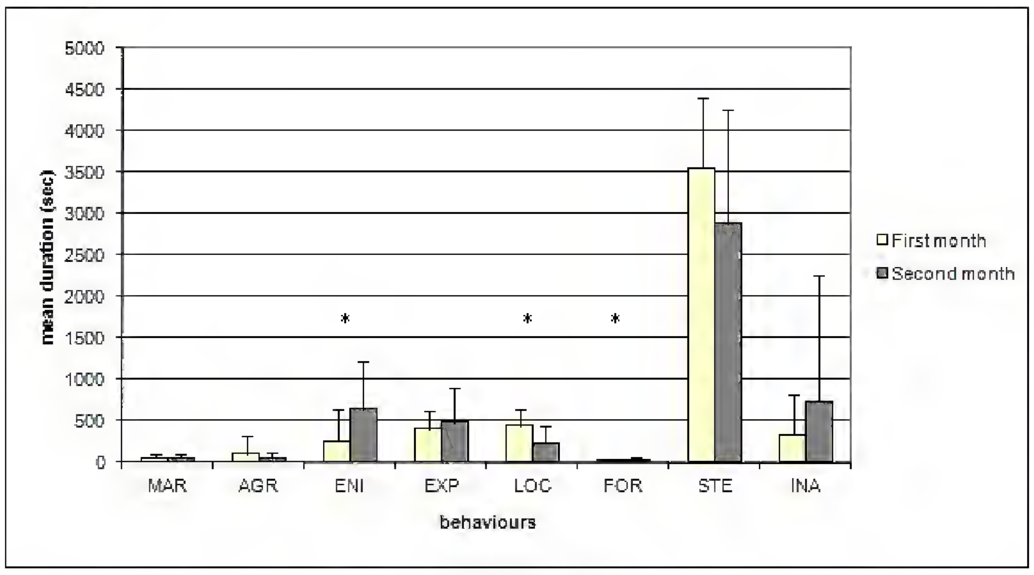

Figure 1. Comparison of different behaviours between the first (12 sessions) and the second month (12 sessions). MAR = Marking; AGR = Aggression; ENI = Envrichment Interaction; EXP = Exploration; LOC = Locomotion; FOR = Foraging; STE = Stereotype; INA = Inactivity. Behavioural mean duration was collected; error bars stand for the standard deviation; * P = 0.05.

"First phase" vs. "last phase" vs. "post phase" six months after arrival of the fossa

The use of the intense enrichment programme for six months, after the fossa's arrival, resulted in an improvement in natural behaviours and reduced maladaptive self-directed behaviours. Based on the analysis of behaviours performed during the "first phase", "last phase", and "post phase" (six months after his arrival), there was a significant change in the performance of abnormal and normal behaviours over six months (One-Sample Test: First Phase, P = 0.0156; Last Phase, P = 0.0156; Post Phase, P = 0.0078). In particular, the fossa showed less stereotypy in the "post phase" compared with the "last phase" and "first phase" (Figure 2). Indeed stereotypy was always more common than normal behaviours in both "first phase" and "last phase"; on the contrary normal behaviours became more common than stereotypy in "post phase".

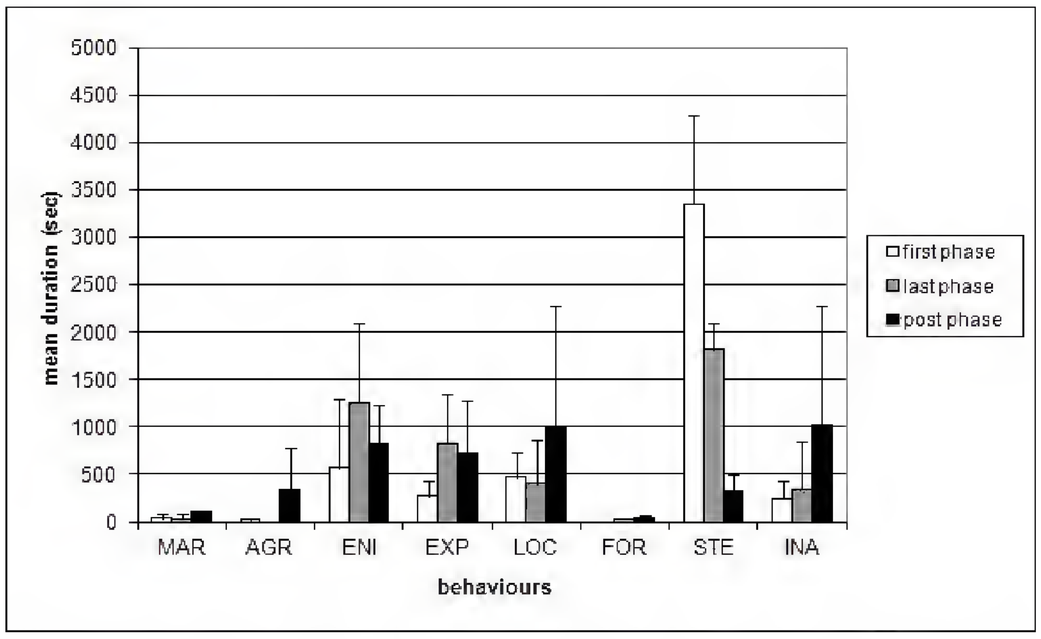

Figure 2. Comparison of different behaviours between the first and last phases (over 2-month period) and the post phase (6 months after his arrival). MAR = Marking; AGR = Aggression; ENI = Enrichment Interaction; EXP = Exploration; LOC = Locomotion; FOR = Foraging; STE = Stereotype; INA = Inactivity. Behavioural mean duration was collected; error bars stand for the standard deviation; * P = 0.05.

After two months of behavioural observations, the fossa's behaviour improved slightly; however, stereotyped and self-injuring behaviours were maintained, despite the intensive enrichment programme. The predatory behaviour of the fossa was not species-specific (Garbutt 2007) since the fossa was not able to find food items when they were hidden in the enclosure. He usually interacted with items for just a few minutes and rubbing different scents on enclosure furniture promoted play only for a few minutes. However, we decided to continue the intense enrichment programme for a longer period (Abello *et al.* 1999).

Behavioural data recorded six months after his arrival showed rare undesirable behaviours or noticeable side effects. Results showed the environmental enrichment curtailed the aberrant behaviour. The fossa was rehabilitated due to an intense enrichment programme that was designed to stimulate all five senses of this carnivore (Allard *et al.* 2003; Kleiman *et al.* 2010). The design of the exhibit was also helpful because it allowed the fossa to perform many species appropriate behaviours.

This case study is an example of how good management of captive animals provide an invaluable support to reach an acceptable level of psychological and physical well-being (Hosey *et al.* 2009). In addition, the present study reports evidence that an appropriate environmental enrichment programme could be a therapeutic tool for managing pathological behaviour in captive animals (Carrasco *et al.* 2009).

Acknowledgements

We are grateful to Dr. Cesare Avesani Zaborra and Dr. Camillo Sandri for encouraging this practice. We also thank Prof. Jacopo Moggi-Cecchi and Prof. Francesco Ferrini for allowing the visit to the Garda Zoological Park by S.V.

References

Abello M., Velasco M., Esteban F. (1999) A training programme for a male gorilla at the Barcelona Zoo. *International Zoo News* 46(7): 418-420.

Allard S.M., Stoinski T.S., Bloomsmith M.A., Maple T.L. (2003) The effects of enrichment structures on captive gorilla behavior. *American Journal of Primatology* 60(1): 95-96.

Barlow D.H., Hersen M. (1984) *Single-case experimental designs: Strategies for studying behavior change* (2nd ed.). New York: Pergamon Press.

Box G., Jenkins G.M., Reinsel G. (1994) *Time series analysis: Forecasting and control* . 3rd edition. Englewood Cliffs, New Jersey: Prentice Hall.

Carrasco L., Colell M., Calvo M., Abello M.T., Velasco M., Posada S. (2009) Benefits of training/playing therapy in a group of captive lowland gorillas (*Gorilla gorilla gorilla*). *Animal Welfare* 18(1): 9-19.

Clubb R., Vichery S., Latham N. (2006) Motivation and motivational explanations for stereotypies. In: *Stereotypic Animal Behaviour: Fundamentals and Application to Welfare 12.* 2nd Edition. Mason, G. and Rushen, J. (Eds.). Trowbridge: Cromwell Press.

Franklin R.D., Allison D.B., Gorman B.S. (1997) Design and analysis of single-case research. Mahwah, New Jersey: Lawrence Erlbaum Associates.

Garbutt N. (2007) *Mammals of Madagascar: a complete guide.* London: A. & C. Black.

Hosey G., Melfi V., Pankhurst S. (2009) *Zoo Animals: behaviour, management, and welfare.* Oxford: Oxford University Press.

Jones W.P. (2003) Single-case time series with Bayesian analysis: A practitioner's guide. *Measurement and Evaluation in Counseling & Development* 36: 28-39.

Kleiman D.G., Thompson K.V., Baer C.K. (2010) *Wild Mammals in Captivity: Principles and Techniques for Zoo Management.* Chicago: University of Chicago Press.

MacDonald D. (2001) *The New Encyclopedia of Mammals.* Oxford and New York: Oxford University Press.

Mallonée J.S., Joslin P. (2004) Traumatic stress disorder observed in an adult wild captive wolf (*Canis lupus*). *Journal of Applied Animal Welfare Science* 7(2): 107-126.

Mason G.J. (1991) Stereotypies: a critical review. *Animal Behaviour* 41: 1015-1037.

Mason G.J., Clubb R., Vickery S., Latham N. (2006) Why and how we should use environmental enrichment to tackle stereotypic behaviour? *Applied Animal Behaviour Science* 102: 163-188.

Nock M.K., Michel B.D., Photos V.I. (2007) Single-case research designs. In: *Handbook of Research Methods in Abnormal and Clinical Psychology* pp.337-350. McKay D. (Ed.). Thousand Oaks, California: Sage.

Young R.J. (2003) *Environmental Enrichment for Captive Animals*. Oxford: Blackwell Publishing.

Shepherdson D.J., Mellen J.D., Hutchins M. (1998) *Second nature: Environmental enrichment for captive animals.* Washington, DC: Smithsonian Institution Press.

Siegel S., Castellan N.J. (1992) *Non parametric Statistics for the Behavioural Sciences.* 2nd edition. London: MacGraw-Hill.

Stewart K.L., Bayne K. (2004) Environmental Enrichment for Laboratory Animals. In: *Laboratory Animal Medicine and Management: 10.* Reuter, J.D. and Suckow, M.A. (Eds.). Ithaca: International Veterinary Information Service.